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Sir:
Transmitted herewith for filing is the utility patent application of inventor(s): Patricia G. Spear and Rebecca I. Montgomery and entitled: HERPES VIRUS ENTRY RECEPTOR PROTEIN.

1. Enclosed are:
   - x — A duplicate copy of this transmittal letter.
   - x — One stamped, self-addressed postcard for the PTO Mail Room date stamp.
   - x — One utility patent application containing pages 1-47, and
     - ___ an executed declaration or oath for the utility patent application including a power of attorney,
     - x an unexecuted declaration or oath for the utility patent application including a power of attorney, and
     - x drawings: ___ 1 copy of ___ sheets of formal drawings, OR
       1 1 copy of 11 sheets of informal drawings, OR
       ___ 1 set of ___ Bristol board sheets of original, formal drawings.
   - ___ A certified copy of a ___ application, No. ___.
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   - ___ Other: ___

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Total Claims	27 - 20	= 7	x $ 11 =	$77	OR	x $ 22 =	$
Independent Claims	4 - 3	= 1	x $ 38 =	$38	OR	x $ 76 =	$
Multiple Dependent Claim Presented			+ $120 =	$	OR	+ $240 =	$
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3. The enclosed utility patent application is related to: ___

Date: July 28, 1995

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HERPES VIRUS ENTRY RECEPTOR PROTEIN

Technical Field of the Invention

The field of this invention is a herpes virus entry receptor (~~HVER~~ HVEM). More particularly, the field of the present invention is recombinant mammalian ~~HVER~~ HVEM, polynucleotides encoding that ~~HVER~~ HVEM, and methods of making recombinant ~~HVER~~ HVEM.

Background of the Invention

Glycosaminoglycan chains on cell surface proteoglycans serve as receptors for the binding of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) to cells. Binding is not sufficient for entry, however: other cell surface components are necessary for virus entry, which occurs by fusion of the virion envelope with a cell membrane. For example, Chinese hamster ovary (CHO) cells express glycosaminoglycan chains to which HSV-1 and HSV-2 can bind, but are resistant to the entry of some HSV strains, particularly HSV-1(KOS).

The present invention is directed to a newly discovered protein that enables herpes simplex virus (HSV) to penetrate into cells and is a previously undiscovered member of the family of receptors designated the tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) family. Members of this family have characteristic repeats of amino acid sequence containing multiple cysteines and serve as receptors for a variety of specific ligands, including but not limited to cytokines. The protein is designated herpes virus entry receptor protein or ~~HVER~~ HVEM.

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By identifying the gene that encodes HVEM ~~HVER~~, by showing that HVEM ~~HVER~~ can mediate the entry of HSV into cells and by performing experiments to define viral and cell factors that influence the ability of HVEM ~~HVER~~ to mediate HSV entry, the inventors have provided the knowledge and biological material required (i) to develop antiviral drugs that can act to block HSV (and perhaps other herpesvirus) entry into cells; (ii) to identify other members of the TNFR/NGFR family (or other cell surface molecules) that can serve as receptors for HSV-1, HSV-2 or other herpesviruses; (iii) to identify the natural ligand for the receptor; and (iv) to develop therapeutic approaches for enhancing or inhibiting action of the ligand on the receptor, depending on the pathologic or beneficial consequences of this action.

Brief Summary of the Invention

In one aspect, the present invention provides an isolated and purified polynucleotide comprising a nucleotide sequence consisting essentially of the nucleotide of SEQ ID NO:1 from about nucleotide position 294 ~~293~~ to about nucleotide position 1142 ~~1189~~; (b) sequences that are complementary to the sequences of (a), and (c) sequences that, when expressed, encode a polypeptide encoded by a sequence of (a). A preferred polynucleotide is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule. A preferred polynucleotide is SEQ ID NO:1.

In another embodiment, a DNA molecule of the present invention is contained in an expression vector. The expression vector preferably further comprises an enhancer-promoter operatively linked to the polynucleotide. In an especially preferred embodiment, the DNA molecule has the nucleotide sequence of SEQ ID NO:1 from about nucleotide position 294 ~~293~~ to about nucleotide position 1142 ~~1189~~.

In another aspect, the present invention provides an oligonucleotide of from about 15 to about 50 nucleotides containing a nucleotide sequence of at least 15 nucleotides that is identical or complementary to a contiguous sequence of a polynucleotide of this invention. A preferred oligonucleotide is an antisense oligonucleotide that is complementary to a portion of the polynucleotide of SEQ ID NO:1.

The present invention also provides a pharmaceutical composition comprising a polypeptide or an antisense oligonucleotide of this invention and a physiologically acceptable diluent.

In another aspect, the present invention provides an ~~HVER~~ HVEM polypeptide of mammalian origin. In one embodiment, that ~~HVER~~ HVEM is an isolated and purified polypeptide of about 300 amino acid residues and comprises the amino acid residue sequence of SEQ ID NO:2. More preferably, an ~~HVER~~ HVEM of the present invention is a recombinant human ~~HVER~~ HVEM.

In another aspect, the present invention provides a process of making ~~HVER~~ HVEM comprising transforming a host cell with an expression vector that comprises a polynucleotide of the present invention, maintaining the transformed cell for a period of time sufficient for expression of the ~~HVER~~ HVEM and recovering the ~~HVER~~ HVEM. Preferably, the host cell is an eukaryotic host cell such as a mammalian cell, or a bacterial cell. An especially preferred host cell is a mammalian ovarian cell. The present invention also provides an ~~HVER~~ HVEM made by a process of this invention. A preferred such ~~HVER~~ HVEM is recombinant human HVER.

The present invention still further provides for a host cell transformed with a polynucleotide or expression vector of this invention. Preferably, the host cell is a mammalian cell such as an ovarian cell.

Brief Description of the Drawings

In the drawings, which form a portion of the specification:

FIG. 1 shows a map of the plasmid (pBEC580) cloned from the cDNA library on the basis of its ability to convert resistant CHO-K1 cells to susceptibility to HSV-1(KOS) infection. The cDNA insert prepared as described in the text was ligated between BstXI and Not I sites in the polylinker region of pcDNAI (shown in the inset).

G1 D1 ~~FIG. 2 shows the nucleotide sequence (SEQ ID NO:1) of the cDNA insert of pBEC580 and amino acid sequence (SEQ ID NO:2) translated from the open reading frame designated HVER. Predicted features of the HVER polypeptide include a signal sequence (dotted underline), two potential sites for the addition of N-linked glycans (bold underline), a hydrophobic region that could potentially span a membrane (underline) and three cysteine-rich repeats characteristic of members of the TNF/NGF receptor family (shaded boxes).~~

FIG. 3 shows a map of the plasmid (pBEC10) produced by transferring the cDNA insert of pBEC580 to the vector, pcDNA3 (shown in the inset). The cDNA insert was excised from pBEC580 by cutting with HindIII and XhoI and was ligated to pcDNA3 that had also been cut with HindIII and XhoI. The position of the cytomegalovirus promoter (P-CMV) is shown and also the position of the selectable marker Neo, along with upstream and downstream sequences required for its expression in eukaryotic cells.

G2 ~~FIG. 4 shows susceptibility of HeLa cells and various CHO cell lines to infection by HSV-1(KOS). The values reported are the optical density at 410 nm. Each point represents the mean of triplicate (panel A) or quadruplicate (panel B) determinations. The individual values were within 10% of the mean. A. HeLa cells (open circles) and~~

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CHO-K1 cells (closed circles). B. CHO cell lines stably transfected with pBEC10, which carries the HVER cDNA [CHO-A3 (closed triangles); CHO-A12 (open squares); CHO-B3 (open triangles); CHO-B9 (closed squares); CHO-B11 (open circles)] and a control cell line stably transfected with the vector pcDNA3 [CHO-C8 (closed circles)].

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FIG. 5 shows replication of three HSV strains in CHO cells stably transfected with HVEM ~~HVER~~ and in control CHO cells and HeLa cells. Cells plated in 6-well plates at about 5 x 10^6 cells per well were inoculated with the virus indicated at 10^8 PFU per well, to ensure that all susceptible cells were synchronously infected. After allowing 2 hr for virus binding and entry, the cells were washed and treated with citrate buffer, pH 3, to inactivate input virus that bound to cells but failed to initiate infection. Culture medium was added and one set of cultures harvested immediately (2 hr after addition of the virus inoculum) for quantitation of infectious virus by plaque assay on Vero cells, to determine the baseline viral titer prior to the appearance of progeny virus (black bars). The remainder of the cultures were harvested at 31 hr for quantitation of viral progeny (diagonal-hatched bars). The values presented represent half the yield from each culture.

G3

FIG. 6 shows CHO-A12 cells that express HSV-1 or HSV-2 gD are resistant to HSV-1(KOS) infection. The results shown are for the amount of plasmid DNA giving maximal interference (1.5 μg per well for the gD-1-expressing plasmid and 2.0 μg per well for the gD-2-expressing plasmid). The control plasmid was used at 1.5 μg per well and the CHO-K1 cells were not transfected. The values given are the means of quadruplicate determinations.

FIG. 7 shows a map of the plasmid (pBL58) expressing the HVEM ~~HVER~~-Fc ~~Ig~~ hybrid protein.

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~~FIGs. 8A and 8B show the nucleotide sequence (SEQ ID NO:6) of pBL58 and the amino acid sequence (SEQ ID NO:7) of the open reading frame encoding HVER-Ig. Features of the HVER ectodomain that were described in FIG. 2 are shown here along with the site at which the HVER sequence is fused to the rabbit IgG heavy chain sequence (the boxed residues are three amino acids inserted at the fusion site due to the EcoRI linker added). The two potential sites for the addition of N-linked glycans in HVER are underlined along with a third site in the IgG sequence.~~

FIG. ~~9~~ ~~8~~ 9 shows a schematic drawing of human ~~HVER~~ HVEM. The protein has characteristics of a typical type I membrane glycoprotein, including an N-terminal signal sequence (diagonal-hatched box) and a membrane-spanning region (cross-hatched box). The protein also has the cysteine-rich repeats characteristic of the TNFR/NGFR family of cell surface receptors. Each repeat has 4-6 cysteine residues (represented by vertical lines).

FIG. ~~10~~ ~~9~~ 10 shows the relative susceptibilities of A12 cells transfected with various gD-expressing plasmids.

Detailed Description of the Invention

I. The Invention

The present invention provides isolated and purified polynucleotides that encode ~~HVER~~ HVEM of mammalian origin, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process of making ~~HVER~~ HVEM using those polynucleotides and vectors, and isolated and purified ~~HVER~~ HVEM.

II. ~~HVER~~ HVEM Polynucleotides

In one aspect, the present invention provides an isolated and purified polynucleotide that encodes an HVEM ~~HVER~~ polypeptide of mammalian origin.

A polynucleotide of the present invention that encodes HVEM ~~HVER~~ is an isolated and purified polynucleotide that comprises a nucleotide sequence consisting essentially of the nucleotide sequence of SEQ ID NO:1 from about nucleotide position 244 ~~293~~ to about nucleotide position 1142 ~~1189~~ of SEQ ID NO:1, (b) sequences that are complementary to the sequences of (a), and (c) sequences that, when expressed, encode a polypeptide encoded by the sequences of (a). A preferred polynucleotide is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule.

~~A nucleotide sequence and deduced amino acid residue sequence of human HVEM HVER are set forth in FIG. 2. The nucleotide sequence of SEQ ID NO:1 in FIG. 2 is a full length DNA clone of human HVER. SEQ ID NO:2 in FIG. 2 is the deduced amino acid residue sequence of that clone.~~

The present invention also contemplates DNA sequences which hybridize under stringent hybridization conditions to the DNA sequences set forth above. Stringent hybridization conditions are well known in the art and define a degree of sequence identity greater than about 70%-80%. The present invention also contemplates naturally occurring allelic variations and mutations of the DNA sequences set forth above so long as those variations and mutations code, on expression, for an HVER of this invention as set forth hereinafter.

As set forth above, SEQ ID NO:1, is a full length cDNA clone of human HVEM ~~HVER~~. As is well known in the art, because of the degeneracy of the genetic code, there are numerous other DNA and RNA molecules that can code for the same polypeptide as those encoded

by SEQ ID NO:1. The present invention, therefore, contemplates those other DNA and RNA molecules which, on expression, encode for the polypeptide encoded by SEQ ID NO:1. Having identified the amino acid residue sequence of HVEM ~~HVER~~, and with knowledge of all triplet codons for each particular amino acid residue, it is possible to describe all such encoding RNA and DNA sequences. DNA and RNA molecules other than those specifically disclosed herein and, which molecules are characterized simply by a change in a codon for a particular amino acid are within the scope of this invention.

A Table of codons representing particular amino acids is set forth below in Table 1.

TABLE 1

First position (5' end)	Second Position T/U	C	A	G	Third position (3' end)
	Phe	Ser	Tyr	Cys	T/U
	Phe	Ser	Tyr	Cys	C
T/U	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
	Leu	Pro	His	Arg	T/U
	Leu	Pro	His	Arg	C
C	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
	Ile	Thr	Asn	Ser	T/U
	Ile	Thr	Asn	Ser	C
A	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
	Val	Ala	Asp	Gly	T/U
	Val	Ala	Asp	Gly	C
G	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

A simple change in a codon for the same amino acid residue within a polynucleotide will not change the structure of the encoded polypeptide. By way of example, it can be seen from SEQ ID NO:1 (see FIG. 2) that a CCT codon for proline exists at nucleotide positions 300-302 ~~239-241~~. It can also be seen from that same sequence, however, that proline can be encoded by a CCC codon (see e.g., nucleotide positions 324-326 ~~323-325~~). Substitution of the latter CCC codon for proline with the CCT codon for proline, or vice versa, does not substantially alter the DNA sequence of SEQ ID NO:1 and results in expression of the same polypeptide. In a similar manner, substitutions of codons for other amino acid residues can be made in a like manner without departing from the true scope of the present invention.

A polynucleotide of the present invention can also be an RNA molecule. A RNA molecule contemplated by the present invention is complementary to or hybridizes under stringent conditions to any of the DNA sequences set forth above. As is well known in the art, such a RNA molecule is characterized by the base uracil in place of thymidine. Exemplary and preferred RNA molecules are mRNA molecules that encode an HVEM ~~HVER~~ of this invention.

The present invention also contemplates oligonucleotides from about 15 to about 50 nucleotides in length, which oligonucleotides serve as primers and hybridization probes for the screening of DNA libraries and the identification of DNA or RNA molecules that encode HVEM ~~HVER~~. Such primers and probes are characterized in that they will hybridize to polynucleotide sequences encoding HVEM ~~HVER~~ or related receptor proteins. An oligonucleotide probe or primer contains a nucleotide sequence of at least 15 nucleotides that is identical to or complementary to a contiguous sequence of an HVEM ~~HVER~~ polynucleotide of the present invention. Thus, where an oligonucleotide probe is 25 nucleotides in length, at least 15 of those nucleotides are identical or complementary to a sequence of contiguous nucleotides of an HVEM ~~HVER~~ polynucleotide of the present invention. Exemplary HVEM ~~HVER~~ polynucleotides of the present invention are set forth above.

A preferred oligonucleotide is an antisense oligonucleotide. The present invention provides a synthetic antisense oligonucleotide of less than about 50 nucleotides, preferably less than about 35 nucleotides, more preferably less than about 25 nucleotides and most preferably less than about 20 nucleotides. An antisense oligonucleotide of the present invention is directed against a DNA or RNA molecule that encodes HVEM ~~HVER~~. Preferably, the antisense oligonucleotide is directed against the protein translational initiation site or the transcriptional start site. In accordance with this preferred embodiment, an antisense molecule is directed against a region of SEQ. ID NO:1 from about nucleotide

position ~~253~~ 254 to about nucleotide position ~~333~~ 334. It is understood by one of ordinary skill in the art that an antisense oligonucleotide can be directed either against a DNA or RNA sequence that encodes a specific target. Thus, an antisense oligonucleotide of the present invention can also be directed against polynucleotides that are complementary to those shown in SEQ. ID NO:1 as well as the equivalent RNA molecules.

Preferably, the nucleotides of an antisense oligonucleotide are linked by pseudophosphate bonds that are resistant to cleavage by exonuclease or endonuclease enzymes. Preferably the pseudophosphate bonds are phosphorothioate bonds. By replacing a phosphodiester bond with one that is resistant to the action of exo-and/or endonuclease, the stability of the nucleic acid in the presence of those enzymes is increased. As used herein, pseudophosphate bonds include, but are not limited to, methylphosphonate, phosphomorpholidate, phosphorothioate, phosphorodithioate and phosphoroselenoate bonds.

An oligonucleotide primer or probe, as well as an antisense oligonucleotide of the present invention can be prepared using standard procedures well known in the art. A preferred method of polynucleotide synthesis is via cyanoethyl phosphoramidite chemistry. A detailed description of the preparation, isolation and purification of polynucleotides encoding human HVEM ~~HVER~~ is set forth below.

Briefly, CHO-K1 cells are resistant to the entry of HSV-1(KOS). The present invention discloses an assay to screen for human cDNAs encoding proteins capable of conferring susceptibility to HSV-1(KOS) infection on the CHO-K1 cells. Control and transfected CHO-K1 cells were exposed to a strain of HSV-1(KOS) that had been modified to express *E. coli* beta-galactosidase, under control of a human cytomegalovirus promoter, immediately after viral entry into a cell. Any transfected cells that became susceptible to HSV-1(KOS) entry expressed beta-galactosidase after infection. Addition of the appropriate beta-

galactosidase substrate (X-gal) caused the infected cells to turn blue. The high level of resistance of the CHO-K1 cells to HSV-1(KOS) infection made it possible to detect very small numbers of cells rendered susceptible to infection by transfection of the human cDNAs.

A commercially obtained unidirectional cDNA library prepared from human HeLa cell mRNA was used for the transfections. The plasmids in this library express human proteins under control of the human cytomegalovirus promoter, after transfection into eukaryotic cells. The cDNA library was purchased from Invitrogen Corp (3985 B Sorrento Valley Blvd., San Diego, CA 92121):

T0130

catalog no.	A950-10
mRNA source	HeLa cells (a human cell line derived from a carcinoma)
primer	oligo dT(Not I)
vector	pcDNAI

This library was constructed using materials produced by Invitrogen according to the following protocol:

mRNA was isolated from the HeLa cells using the Invitrogen FastTrack® mRNA Isolation Kit. The mRNA was copied by AMV reverse transcriptase, using an oligo dT(NotI) primer, to produce the first strand of DNA. The sequence of this primer is 5'- d PO_4[AACCCGGCTCGAGCGGCCGCT_{18}]-3' (SEQ ID NO:3). The underlined sequence is the NotI site used in a later step for cleavage of the cDNA and its insertion into the vector in a directional fashion.

The product was converted to double-stranded DNA by DNA polymerase in combination with RNaseH, and *E. coli* DNA ligase. Any sticky (single-stranded) ends were made blunt (filled in) by use of T4 polymerase. A BxtXI/EcoRI adapter was added to the ends by blunt-

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end ligation. The sequence of the adapter is: GAATTCCACCACACTTAAGGTG (SEQ ID NO.:4). The cDNA was cut with BstXI and NotI and cloned directionally by sticky-end ligation into pcDNAI, which had also been cut with BstXI and Not I.

The plasmids were used to transform *E. coli* strain MC1061/P3. The number of primary recombinant plasmids was estimated to be about 1.5 x 10^6. The number of colonies in the amplified library was 4.5 x 10^7 per ml. The estimated size range of the inserts was 0.9 kb to 1.6 kb.

1.5 x 10^7 bacteria were plated (1.5 x 10^5 bacteria per large Petri plate for a total of 100 plates) to allow the growth of bacterial colonies. The colonies were scraped from each plate to yield one pool of bacteria from each plate. Samples of the 100 bacterial pools were mixed to yield 10 mixtures of 10 pools each. Plasmid DNA was extracted from each mixture of pools by standard means.

Each plasmid DNA mixture was prepared with LipofectAMINE™ (GibcoBRL), according to the manufacturer's directions, for transfection into Chinese hamster ovary cells, strain K1 (CHO-K1).

To determine whether any of the transfected cells became susceptible to HSV-1(KOS) infection, the transfected cells and control cells (untransfected or transfected with irrelevant DNA) were exposed to a mutant form of HSV-1(KOS) at an input dose sufficiently high to infect all susceptible cells. This mutant is deleted for one of the essential glycoproteins, gL, and must be propagated on gL-expressing Vero cells. The virus produced on the gL-expressing cells is fully infectious but can undergo only one round of replication because defective virus is produced in non-complementing cells. The gL open reading frame was

replaced by the *E. coli* lacZ gene, downstream of the strong cytomegalovirus promoter. The lacZ gene encodes beta-galactosidase.

After exposure to virus for several hours, the transfected CHO-K1 cells were fixed and incubated with the beta-galactosidase substrate, X-gal. Susceptible cells were readily identified by their blue color resulting from conversion of the substrate to an insoluble blue preceipitate by the beta-galactosidase expressed after entry of the mutant HSV-1(KOS).

DNA from one mixture of ten pools was found to be positive for ability to convert some of the transfected CHO-K1 cells to susceptibility. Each of the ten bacterial pools in this mixture was tested separately by extracting plasmid DNA and repeating steps set forth above. Pool 82 was found to be positive.

Bacterial pool 82 was itself divided into 100 subpools as described above. It was found that subpool 53 was positive. The bacteria in subpool 53 were plated and 900 individual clones were picked and grown up. Plasmids DNAs were extracted from each of the clones for testing. Clone 580 was found to be positive. Clone 580 was designated pBEC580. A map of this plasmid is shown in FIG. 1.

The nucleotide sequence of the cDNA insert of pBEC580 was determined by use of the Sequenase® kit (US Biochemical Corp) according to the manufacturer's instructions.

The PCgene suite of software from Intelligenetics, Inc. was used to analyze the nucleotide sequence. As shown in FIG. 2, one open reading frame was found in the correct orientation. The protein encoded in this open reading frame was designated a herpesvirus entry receptor protein (~~HVER~~ HVEM) and was found by sequence analysis to have properties of a type I membrane glycoprotein. Shown in FIG. 2 are 1) the

predicted signal peptide that could direct translocation of the nascent peptide across membranes of the rough endoplasmic reticulum; 2) two sites that are signals for the addition of N-linked carbohydrate; and 3) a hydrophobic region that is predicted to be a membrane-spanning region, adjacent to a very basic region which could serve to anchor the protein in a membrane.

The blastp and blastn programs were used to search databases maintained by the National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health, in Bethesda, MD, for proteins or nucleotide sequences that might be identical to, or related to, those of the cDNA insert. The blastp program was used to search for HVEM ~~HVER~~-related protein sequences in the database updated daily that contains non-redundant protein sequences from five component databases (Brookhaven Protein Data Bank, the SWISS-PROT database, The PIR database, the coding sequence translations from the GenBank databases and two other databases that contain cumulative weekly or daily updates, respectively, of the SWISS-PROT database and the translations from Gen Bank).

This search failed to detect any closely related proteins, indicating that HVEM ~~HVER~~ has not been previously described. The blastp program identified about 30 proteins that share a characteristic sequence motif with HVEM ~~HVER~~, namely three or more cysteine-rich repeats with a characteristic pattern of 6 cysteine residues. These other proteins that are related to HVEM ~~HVER~~ by this motif are all members of the TNF/NGF receptor family. They encode membrane receptors that can be triggered by the binding of specific ligands to activate specific pathways important to cell survival, apoptosis or induced protective responses against infectious agents or trauma.

The blastn program identified two entries in the DNA database (the combined non-redundant database consisting of nucleotide

sequence entries from the Brookhaven Protein Data Bank, GenBank, the EMBL Data Library and cumulative daily updates of the GenBank and EMBL databases) that provide partial nucleotide sequence information for cDNAs that are very closely related to the cDNA encoding ~~HVER~~ HVEM. One entry (locus HSC0BG042) provides partial sequence that is closely related to sequence in the 3' non-coding region of the ~~HEVR~~ HVEM cDNA. The other entry (locus HSC0BG041) provides partial sequence that is closely related to sequence in the 5' non-coding region and extending 43 amino acids into the N-terminal region of the ~~HVER~~ HVEM open reading frame, but not extending into the TNF/NGF receptor motifs.

The cDNA insert was transferred to another vector, pcDNA3, which carries a selectable marker (the neomycin gene) that can be used to isolate cell lines stably carrying the plasmid. Cells that carry and express this gene are resistant to the toxic effects of a drug called G418. The cDNA insert of pBEC580 was excised by cutting with HindIII and XhoI and the insert was ligated to pcDNA3, which had also been cut with HindIII and XhoI, to produce the new plasmid called pBEC10. A map of pBEC10 is shown in FIG. 3.

CHO-K1 cells were transfected with pBEC10 or pcDNA3 and, after about 48 hours, incubated with medium containing G418. Only cells carrying the plasmid (with the Neo marker) were able to survive.

Several stably transformed colonies of cells were isolated after transfection with each plasmid and were cloned. None of the clones obtained with pcDNA3 were susceptible to HSV-1(KOS) infection. About half of the clones obtained with pBEC10 were susceptible (the resistant clones may not have been able to express the protein encoded in the cDNA insert). Cells plated in 96-well dishes, at densities ranging from 10^4 to 5×10^4 cells per well, were exposed to HSV-1(KOS)gL86 in the quantities indicated. At 6 hr after the addition

of virus, the cells were solubilized with detergent and beta-galactosidase substrate added to assess the efficiency of viral entry. Expression of beta-galactosidase signals that the virus has entered a cell and the amount of enzyme produced is proportional to the number of infected cells, at least until plateau values of beta-galactosidase activity are achieved. FIGs. 4 and 5 show that CHO-K1 parental cells and CHO cells transfected with the control plasmid, pcDNA3, are resistant ~~toHSV-~~ to HSV-1(KOS) infection whereas the cells transfected with, and stably carrying pBEC10, are susceptible to HSV-1(KOS) infection.

Although the cells transfected with the HVEM ~~HVER~~ cDNA are fully susceptible to infection by HSV-1(KOS), they are resistant to infection by a mutant of HSV-1(KOS), designated HSV-1(KOS)rid1, that differs from parental virus only by an amino acid substitution in the viral envelope glycoprotein gD. This indicates that gD, at least in part, determines the ability of virus to use HVEM ~~HVER~~ for entry. Because HSV-1(KOS) expressing the mutant form of gD can infect human cells almost as efficiently as parental HSV-1(KOS), there must be cell surface molecules expressed in human cells, in addition to HVEM ~~HVER~~, that can be used for entry.

CHO-A12 cells in 6-well plates were transfected with plasmids that express HSV-1 gD (pRE4) or HSV-2 gD (pWW65) under control of the Rous sarcoma virus promoter or with a control plasmid consisting of the vector with no insert (pdH). These plasmids were obtained from G. Cohen and R. Eisenberg (Univ. of Pennsylvania). Transfection was done with the LipofectAMINE™ reagent (GibcoBRL) using plasmid quantities ranging from 0.5 to 2.5 μg per well. At 24 hr after transfection, the cells were replated in 96-well plates and, 12 hr later, were exposed to HSV-1(KOS)gL86 to assess the susceptibility of the cells to infection.

Transfection of ~~HVER~~ HVEM-expressing CHO cells with a plasmid that expresses wild-type gD (either the HSV-1 or HSV-2 forms of gD) confers resistance to infection by HSV-1(KOS) (See FIG. 6). This is an interference activity of gD that has been previously described. When gD is expressed by the cell, it can render a susceptible cell resistant to HSV-1 infection, possibly by sequestering a cell surface receptor needed for HSV-1 entry. The fact that gD expression renders the ~~HVER~~ HVEM-expressing cells resistant to HSV-1(KOS) infection suggests that there may be a direct physical interaction between gD (both the HSV-1 and HSV-2 forms) and ~~HVER~~ HVEM.

Table 2 below lists the cell lines obtained and summarizes some of their properties:

Table 2.

Cell line	Plasmid used for transfection	Susceptible to infection by:			
		HSV-1(KOS)	HSV-1(KOS)rid1	HSV-1(F)	HSV-2(333)
Cell lines obtained from others or from culture collections:					
HeLa (human)	None	Yes	Yes	Yes	Yes
Hep-2 (human)	None	Yes	Yes	Yes	Yes
CHO-K1 (hamster)	None	No	No	Partially	Yes
New cell lines					
CHO-A3	pBEC10	Yes	N.T.[a]	N.T.[a]	N.T.[a]
CHO-A12[b]	pBEC10	Yes	No[c]	Yes	Yes
CHO-B3	pBEC10	partially	N.T.[a]	N.T.[a]	N.T.[a]
CHO-B9	pBEC10	Yes	N.T.[a]	N.T.[a]	N.T.[a]
CHO-B11	pBEC10	Yes	N.T.[a]	N.T.[a]	N.T.[a]
CHO-C8	pcDNA3	No	No[c]	N.T.[a]	N.T.[a]

[a] N.T.--Not tested.

[b] When the A12 cells were transfected with plasmids expressing HSV-1 or HSV-2 gD, they became resistant to HSV-1(KOS) infection.

[c]CHO-K1 cells are slightly more susceptible to infection by HSV-1(KOS)rid1 than by parental HSV-1(KOS) but the expression of ~~HVER~~ HVEM in the transfected cells does not enhance susceptibility of the cells to

HSV-1(KOS)rid1, in marked contrast to the results obtained with HSV-1(KOS).

Southern blots were done with digests of DNA from three human cell lines (Hep-2, HeLa and HT1080), one monkey cell line (Vero), the Chinese hamster overy cell line used for cloning HVEM ~~HVER~~ (CHO-K1) and two of the CHO cell lines stably tranfected with pBEC10 (CHO-A12 and CHO-B9). The probes used to detect DNA fragments with homology to HVEM ~~HVER~~ were an EcoRI fragment of the HVEM ~~HVER~~ cDNA insert that includes most of the insert and a smaller PvuII fragment that includes only the 3' end of the HVEM ~~HVER~~ open reading frame and some of the on-coding sequence downstream. The results showed that: (i) all three human cell lines contain DNA homologous to HVEM ~~HVER~~ with fragment sizes that are the same for all three cell lines in a single digest (different digests yield hybridizable bands of different sizes but the DNAs from three cell lines are indistinguishable); (ii) only a subset of the human DNA fragments that hybridize to the larger EcoRI fragment also hyubridize to the smaller PvuII fragment; (iii) the monkey cells contain weakly hybridizable DNA fragments of different sizes from those found in the human DNAs; (ic) the parental CHO-K1 cells contain no hybridizable DNA fragments; (v) the stably transfected cell lines (CHO-A12 and CHO-B9) contain DNA homologous to HVEM ~~HVER~~ as predicted.

The results obtained with the human, monkey and Chinese hamster DNAs confirm that HVEM ~~HVER~~ is encoded by a human cDNA and indicate that the human HVEM ~~HVER~~ gene is probably a single-copy gene with multiple introns and exons, perhaps extending over a large stretch of DNA. The results also indicate that monkey cells have a gene related to human HVEM ~~HVER~~. If Chinese hamster cells have an HVEM ~~HVER~~ gene, its sequence has diverged too much to be detected by a human HVEM ~~HVER~~ probe.

Poly-adenylated RNAs extracted from various human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas) were obtained from Clontech, Inc., as samples that had already been fractionated by electrophoresis and transferred to a membrane. The membrane was used for hybridization with ~~the~~ the larger ~~EcoRi~~ EcoRI probe mentioned above (almost the entire ~~HVER~~ HVEM cDNA insert). The results showed that there were variable amounts of RNA homologous to ~~HVER~~ HVEM in all the samples. The largest amounts were found in lung and kidney. The sizes of the bands were about 1.8 and 3.8 kb. The ~~HVER~~ HVEM cDNA insert claimed in the application is about 1.8 kb.

III. ~~HVER~~ HVEM Polypeptides

In another aspect, the present invention provides an ~~HVER~~ HVEM polypeptide of mammalian origin. An ~~HVER~~ HVEM of the present invention is a polypeptide of about 300 amino acid residues. Preferably, an ~~HVER~~ HVEM is a human ~~HVER~~ HVEM. A human form of ~~HVER~~ HVEM is shown in SEQ ID NO:2. Thus, human ~~HVER~~ HVEM can be defined as a polypeptide of about ~~300~~ 293 or less amino acid residues comprising the amino acid residue sequence of SEQ ID NO:2.

The present invention also contemplates amino acid residue sequences that are substantially duplicative of the sequences set forth herein such that those sequences demonstrate like biological activity to disclosed sequences. Such contemplated sequences include those sequences characterized by a minimal change in amino acid residue sequence or type (e.g., conservatively substituted sequences) which insubstantial change does not alter the basic nature and biological activity of ~~HVER~~ HVEM.

It is well known in the art that modifications and changes can be made in the structure of a polypeptide without substantially altering the biological function of that peptide. For example, certain amino acids can be substituted for other amino acids in a given

polypeptide without any appreciable loss of function. In making such changes, substitutions of like amino acid residues can be made on the basis of relative similarity of side-chain substituents, for example, their size, charge, hydrophobicity, hydrophilicity, and the like.

As detailed in United States Patent No. 4,554,101, incorporated herein by reference, the following hydrophilicity values have been assigned to amino acid residues: Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4). It is understood that an amino acid residue can be substituted for another having a similar hydrophilicity value (e.g., within a value of plus or minus 2.0) and still obtain a biologically equivalent polypeptide.

In a similar manner, substitutions can be made on the basis of similarity in hydropathic index. Each amino acid residue has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those hydropathic index values are: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Ser (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gln (-3.5); Asp (-3.5); Asn (-3.5); Lys (-3.9); and Arg (-4.5). In making a substitution based on the hydropathic index, a value of within plus or minus 2.0 is preferred.

A comparison of the amino acid sequence SEQ ID NO:2 with the protein databases maintained at the National Library of Medicine (NIH) and with a computer program designed to detect functional motifs in proteins revealed that ~~HVER~~ HVEM has not previously been described, that it is not closely related to other proteins in the database, but that it has three copies of a cysteine-rich motif found in members of the TNFR/NGFR family.

heavy chain fragment. This latter fragment was prepared for the ligation by using PCR technology to insert an EcoRI site just upstream of the following rabbit sequence (ACAAGACCGTTGCACCCTC) (SEQ ID NO:5). Cleavage at this EcoRI site, followed by filling-in, permitted blunt-end ligation to the PvuII site of ~~HVER~~ HVEM so that the two open reading frames were joined in the same reading frame. The 3' end of the rabbit cDNA insert was cut with PstI and joined to the HindIII site of pGEM4 by blunt-end ligation (the PstI cut end was trimmed and the HindII cut end was filled in). The vector sequences are from pGEM4 and include sequences extending to a unique NheI site that was joined by sticky-end ligation to an SpeI site adjacent to the cytomegalovirus promoter (P-CMV) from pcDNAneo. The other end of the P-CMV region was cut with HindIII and is joined to the HindIII site at the top of the map (FIG. 7)

Expression of the hybrid protein has been demonstrated both in Vero cells and in CHO-K1 cells. The protein is secreted into the medium, as predicted, since it should have a signal sequence for translocation into the cell's secretory pathway but has no membrane-spanning region to anchor it to a membrane. The hybrid protein is readily detected on Western blots by use of commercially available antibodies specific for the constant regions of rabbit IgG.

Treatment of the hybrid protein with various glycosidases (endoH, endoF and endoO) has revealed that the protein carries N-linked glycans of the complex type, which is characteristic for secreted proteins, and also carries O-linked glycans, as predicted. This hybrid protein is used to screen for mouse monoclonal antibodies specific for ~~HVER~~ HVEM and to identify HSV proteins with which it might interact.

(510) The hybrid gene is contained in two different expression plasmids, the latter of which contains a selectable marker for obtaining transformed cells that stably carry the plasmid. Transfection of these

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including pGEM3, pGEM4 and pcDNAneo. Starting from the HindIII site of pBL58, part of the polylinker from pGEM3 (HindIII site to XbaI site) was linked to a sticky end created by cutting the ~~HVER~~ HVEM insert with NheI about 37 nucleotides upstream of the ~~HVER~~ HVEM start codon. Another cleavage of the ~~HVER~~ HVEM insert at a PvuII site within the open reading frame created a blunt end that was blunt-end ligated to the rabbit IgG heavy chain fragment. This latter fragment was prepared for the ligation by using PCR technology to insert an EcoRI site just upstream of the following rabbit sequence (ACAAGACCGTTGCACCCTC) (SEQ ID NO:5). Cleavage at this EcoRI site, followed by filling-in, permitted blunt-end ligation to the PvuII site of ~~HVER~~ HVEM so that the two open reading frames were joined in the same reading frame. The 3' end of the rabbit cDNA insert was cut with PstI and joined to the HindIII site of pGEM4 by blunt-end ligation (the PstI cut end was trimmed and the HindII cut end was filled in). The vector sequences are from pGEM4 and include sequences extending to a unique NheI site that was joined by sticky-end ligation to an SpeI site adjacent to the cytomegalovirus promoter (P-CMV) from pcDNAneo. The other end of the P-CMV region was cut with HindIII and is joined to the HindIII site at the top of the map ~~(FIG. 7)~~ (See SEQ ID No:6, FIGs. 8A and 8B) ~~(See SEQ ID NO:6, FIGs. 8A and 8B)~~.

~~8A and 8B~~ Expression of the hybrid protein (SEQ. ID. NO:7, FIGs. 8A and 8B ~~8A aand 8B~~) has been demonstrated both in Vero cells and in CHO-K1 cells. The protein is secreted into the medium, as predicted, since it should have a signal sequence for translocation into the cell's secretory pathway but has no membrane-spanning region to anchor it to a membrane. The hybrid protein is readily detected on Western blots by use of commercially available antibodies specific for the constant regions of rabbit IgG.

Treatment of the hybrid protein with various glycosidases (endoH, endoF and endoO) has revealed that the protein carries N-linked glycans of the complex type, which is characteristic for secreted

proteins, and also carries O-linked glycans, as predicted. This hybrid protein is used to screen for mouse monoclonal antibodies specific for HVEM ~~HVER~~ and to identify HSV proteins with which it might interact.

The hybrid gene is contained in two different expression plasmids, the latter of which contains a selectable marker for obtaining transformed cells that stably carry the plasmid. Transfection of these plasmids into cells has revealed expression of a hybrid polypeptide of molecular weight approximately 60,000 after dissociation into its component chains.

This hybrid polypeptide, designated HVEM ~~HVER~~/Fc, carries N-linked glycans and is expressed as a dimer held together by disulfide bonds (this is characteristic of hybrid proteins prepared with IgG domains that can dimerize to form the Fc region). Commercially available antibodies specific for rabbit IgG were used to detect HVEM ~~HVER~~/Fc in Western blots and in ELISA assays.

The observed apparent size of the hybrid protein is similar to the size predicted, provided the predicted molecular weight includes about 10,000 for the added carbohydrate.

Evidence has been obtained that HVEM ~~HVER~~ is only one of several cell surface receptors that can mediate the entry of HSV-1 and HSV-2 into cells and that functional use of HVEM ~~HVER~~ (and perhaps other receptors) is determined by the structure of the virion envelope glycoprotein gD. A mutant of HSV-1(KOS), designated HSV-1(KOS)rid1 has a single amino acid substitution in gD that confers resistance to gD-mediated interference with HSV infection and alters slightly the ability of this virus, relative to the wild-type parental strain, to penetrate into various cell types including CHO cells and human cells. By use of a mutant strain of HSV-1(KOS) that is deleted for gD and complemented by replication in cells expressing either the wild-type or mutant form of

gD it has been shown that ~~HVER~~ HVEM expression renders CHO cells fully susceptible to infection by virus carrying wild-type gD but not to infection by virus carrying mutant gD, despite the fact that both viruses could infect human cells with nearly equal efficiency. The implications of this result are several-fold.

First, the result shows that the structure of gD determines whether ~~HVER~~ HVEM can be used as a receptor for entry, raising the possibility of a direct physical interaction. This is consistent with knowledge that gD is one of at least four envelope glycoproteins required for HSV entry. Second, although ~~HVER~~ HVEM is expressed in cultured human cells, such as HeLa cells (the cDNA library used was prepared from HeLa cells), there must be other receptors expressed in human cells that can facilitate the entry of HSV-1(KOS) carrying the mutant form of gD. Third, because CHO-K1 cells are so resistant to HSV-1(KOS) carrying the rid1 form of gD, it is possible to use the gD-negative mutant of HSV-1(KOS), which expresses beta-galactosidase and can be complemented with the rid1 form of gD, to screen for expression of the human gene or genes that can facilitate the entry of HSV-1(KOS)rid1 into CHO-K1 cells.

The possibility exists that several members of the TNFR/NGFR family can serve as receptors for entry of HSV-1, HSV-2 or other herpesviruses, and that the particular receptor favored by a given herpesvirus or strain is determined at least in part by the structure of gD.

Expression of ~~HVER~~ HVEM in CHO-K1 cells significantly enhances the entry of at least two HSV-1 strains. Because the original CHO cells are fully susceptible to entry of the HSV-2 strains tested, it is not possible to assess directly whether ~~HVER~~ HVEM has any effect on HSV-2 entry into CHO-K1 cells. Cells expressing HSV gD become resistant, however, to HSV-1 and HSV-2 infection, and also to infection with related alphaherpesviruses, because of a block to penetration (binding is

unimpaired by gD expression). This phenomenon has been called gD-mediated interference

The fact that HSV-1 gD can interfere with infection by HSV-1, HSV-2 or other herpesviruses implies that all the herpesviruses may use an overlapping set of receptors for entry. Transient expression of gD in CHO cells already expressing HVEM ~~HVER~~ renders the cells resistant to HSV-1(KOS) entry. Both the HSV-2 and HSV-1 forms of wild-type gD are able to interfere with ability of HSV-1(KOS) to use HVEM ~~HVER~~ for entry, suggesting that both forms can interact with HVEM ~~HVER~~ for interference and possibly also for entry. As predicted from the hypothesis about the mechanism of interference, the rid1 form of gD is impaired in ability to mediate interference in HVEM ~~HVER~~-expressing CHO cells (consistent with the finding that virus carrying the rid1 form of gD is impaired in ability to enter HVEM ~~HVER~~-expressing CHO cells).

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The interference activity of gD can be quantitated by transfecting gD-expressin gplasmids into HVEM ~~HVER~~-expressing CHO cells and then exposing the cells to HSV-1(KOS)gL86 to determine whether the cells are susceptible or resistant to viral entry. This provides an assay for testing the interference activity of various fD mutants, in order to define the structural features of fD that are required for interference. A number of mutant forms of gD produced by others (G. Cohen and R. Eisenberg of the Univ. of Pennsylvania) have already been tested by others to determine whether the mutations alter gD function in the virion (function required for viral entry into cells). These same mutant forms of gD (provided by G. Cohen and R. Eisenberg) are being tested for their interference actibity. Results obtained to date are summarized in FIG. 10. CHO-A12 cells, which stably express HVEM ~~HVER~~, were plated on 6-well dishes and transfected with one of several plasmids that express different forms of gD. At 24 hr after transfection, the cells were replated in 96-well plates and 12 hr later they were exposed to HSV-1(KOS)gL86 at several concentrations. At 6 hr after adding virus, the cells were

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solubilized and β-galactosidase substrate was added. The colored product was quantitated by spectromophotometry. Selecting the values obtained (OD410) at a dose of virus where the amount of virus added was directly proportional to the amount of β-galactosidase detected, ~~teh~~ the data were normalized for comparison by dividing the values obtained for cells transfected with a gD-expressing plasmid by the value obtained for cells transfected with a control plasmid (control). The forms of gD expressed by the various plasmids were wild-type gD-1 (pRE4), which is 369 amino acids in length, wild-type gD-2 (pWW65), mutant gD-1 deleted for amino acids 196-207 (pWW13), mutant gD-1 deleted for amino acids 234-244 (pWW17), mutant gD-1 deleted for amino acids 194-287 (pWW49), mutant gD-1 deleted for amino acids 234-287 (pWW52), mutant gD-1 deleted for amino acids 208-287 (pWW61), mutant gD-1 with a substitution that replaces Glu with Asp at position 63 (pWW62), mutant gD-1 deleted for amino acids 338-369 (pWW63) and mutant gD-1 with a substitution that replaces Gln with Pro at position 27 (pMW13). Low (-galactosidase activity implies that the transfected gD had interference activity; high activity indicates that the transfected gD had reduced or no activity. All plasmids used except pMW13 were obtained from G. Cohen and R. Eisenberg (Univ. of Pennsylvania). The results indicate that deletions or alterations of gD between the middle and membrane-spanning region of the molecule eliminate interference activity whereas deletion of the cytoplasmic tail of gD and an amino acid substitution at position 63 are without effect. An amino acid substitution at position 27 (the rid1 mutation) reduces, but does not eliminate, interference activity. From the results obtained to date, it appears that alterations affecting the function of gD in infectivity also affect its function in interference. This is consistent with the hypothesis that gD interference results from competition between cell-associated gD and virion-associated gD for a common target, possibly HVEM ~~HVER~~.

An HVEM ~~HVER~~ polypeptide of the present invention has numerous uses. By way of example, such a polypeptide can be used in a

screening assay for the identification of drugs or compounds that inhibit or augment the action of HVEM ~~HVER~~ (e.g., agonist and antagonist to HSV entry into a cell). A screening assay for the identification of such compound, therefore, can be established whereby the ability of a compound to alter the action of HVEM ~~HVER~~ can be determined by exposing cells to HSV in the presence of a polypeptide of the present invention and varying amounts of compounds suspected of inhibiting the activity of HVEM ~~HVER~~.

The hybrid protein HVEM ~~HVER~~/Fc is being used to immunize rabbits for the production of polyclonal antisera specific for the HVEM ~~HVER~~ portion of the molecule. In addition the hybrid protein is used to screen for hybridomas secreting antibodies specific for the HVEM ~~HVER~~ portion (the mice were immunized with HVEM ~~HVER~~-expressing CHO cells). The hybrid protein is used to determine whether a physical interaction between the hybrid protein and gD or other viral proteins can be detected. The hybrid protein also has use in screening expression cDNA libraries for natural ligands of HVEM ~~HVER~~ and screening compounds for inhibitors of the interaction between HSV virions and HVEM ~~HVER~~.

In addition, an HVEM ~~HVER~~ polypeptide of the present invention can be used to produce antibodies that immunoreact specifically with HVEM ~~HVER~~. Means for producing antibodies are well known in the art. An antibody directed against HVER can be a polyclonal or a monoclonal antibody.

Antibodies against HVEM ~~HVER~~ can be prepared by immunizing an animal with an HVEM ~~HVER~~ polypeptide of the present invention. Means for immunizing animals for the production of antibodies are well known in the art. By way of an example, a mammal can be injected with an inoculum that includes a polypeptide as described herein above. The polypeptide can be included in an inoculum alone or conjugated to a carrier protein such as keyhole limpet hemocyanin (KLH). The

polypeptide can be suspended, as is well known in the art, in an adjuvant to enhance the immunogenicity of the polypeptide. Sera containing immunologically active antibodies are then produced from the blood of such immunized animals using standard procedures well known in the art.

The identification of antibodies that immunoreact specifically with Hvem ~~HVER~~ is made by exposing sera suspected of containing such antibodies to a polypeptide of the present invention to form a conjugate between antibodies and the polypeptide. The existence of the conjugate is then determined using standard procedures well known in the art.

An Hvem ~~HVER~~ polypeptide of the present invention can also be used to prepare monoclonal antibodies against Hvem ~~HVER~~ and used as a screening assay to identify such monoclonal antibodies. Monoclonal antibodies are produced from hybridomas prepared in accordance with standard techniques such as that described by Kohler et al. (Nature, 256:495, 1975). Briefly, a suitable mammal (e.g., BALB/c mouse) is immunized by injection with a polypeptide of the present invention. After a predetermined period of time, splenocytes are removed from the mouse and suspended in a cell culture medium. The splenocytes are then fused with an immortal cell line to form a hybridoma. The formed hybridomas are grown in cell culture and screened for their ability to produce a monoclonal antibody against Hvem ~~HVER~~. Screening of the cell culture medium is made with a polypeptide of the present invention.

IV. Method of Making Hvem ~~HVER~~

In another aspect, the present invention provides a process of making Hvem ~~HVER~~. In accordance with that process, a suitable host cell is transformed with a polynucleotide of the present invention. The transformed cell is maintained for a period of time sufficient for expression of the Hvem ~~HVER~~. The formed Hvem ~~HVER~~ is then recovered.

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Means for transforming host cells in a manner such that those cells produce recombinant polypeptides are well known in the art. Briefly, a polynucleotide that encodes the desired polypeptide is placed into an expression vector suitable for a given host cell. That vector can be a viral vector, phage or plasmid. In a preferred embodiment, a host cell used to produce HOEM ~~HVER~~ is an eukaryotic host cell and an expression vector is an eukaryotic expression vector (i.e., a vector capable of directing expression in a eukaryotic cell). Such eukaryotic expression vectors are well known in the art.

In another embodiment, the host cell is a bacterial cell. An especially preferred bacterial cell is an *E. coli*. Thus, a preferred expression vector is a vector capable of directing expression in *E. coli*.

A polynucleotide of an expression vector of the present invention is preferably operatively associated or linked with an enhancer-promoter. A promoter is a region of a DNA molecule typically within about 100 nucleotide pairs in front of (upstream of) the point at which transcription begins. That region typically contains several types of DNA sequence elements that are located in similar relative positions in different genes. As used herein, the term "promoter" includes what is referred to in the art as an upstream promoter region or a promoter of a generalized RNA polymerase transcription unit.

Another type of transcription regulatory sequence element is an enhancer. An enhancer provides specificity of time, location and expression level for a particular encoding region (e.g., gene). A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell that contains one or more transcription factors that bind to that enhancer. Unlike a promoter, an enhancer can function when located at variable distances from a transcription start site so long as the promoter is present.

As used herein, the phrase "enhancer-promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer promoter is operatively linked to a coding sequence that encodes at least one gene product. As used herein, the phrase "operatively linked" or its grammatical equivalent means that a regulatory sequence element (e.g. an enhancer-promoter or transcription terminating region) is connected to a coding sequence in such a way that the transcription of that coding sequence is controlled and regulated by that enhancer-promoter. Means for operatively linking an enhancer-promoter to a coding sequence are well known in the art.

An enhancer-promoter used in an expression vector of the present invention can be any enhancer-promoter that drives expression in a host cell. By employing an enhancer-promoter with well known properties, the level of expression can be optimized. For example, selection of an enhancer-promoter that is active in specifically transformed cells permits tissue or cell specific expression of the desired product. Still further, selection of an enhancer-promoter that is regulated in response to a specific physiological signal can permit inducible expression.

A coding sequence of an expression vector is operatively linked to a transcription terminating region. RNA polymerase transcribes an encoding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA). Enhancer-promoters and transcription-terminating regions are well known in the art. The selection of a particular enhancer-promoter or transcription-terminating region will depend, as is also well known in the art, on the cell to be transformed.

A clone of the human form of HVEM ~~HVER~~ was identified by DNA sequence analysis as set forth above. This clone was used in all subsequent expression studies. HVEM ~~HVER~~ was expressed in CHO-K1 cells under the control of a human cytomegalovirus promoter.

Expression vectors containing the encoding DNA sequence for all or a portion of human HVEM ~~HVER~~ are designated pBEC580, pBEC10, and pBL58. Both vectors were deposited, under the terms of the Budapest Treaty, on July 28, 1995 in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, and have been assigned ATCC Accession Nos: 97236 (pBEC580), 97235 (pBEC10), and 97237 (pBL10).

The present invention also contemplates a host cell transformed with a polynucleotide or expression vector of this invention. Means for transforming cells and polynucleotides and expression vectors used to transform host cells are set forth above. Preferably, the host cell is an eukaryotic host cell such as a mammalian cell or a prokaryotic cell such as an *E. coli*.

V. Pharmaceutical Compositions

The present invention also provides a pharmaceutical composition comprising a polypeptide or a polynucleotide of this invention and a physiologically acceptable diluent.

In a preferred embodiment, the present invention includes one or more antisense oligonucleotides or polypeptides, as set forth above, formulated into compositions together with one or more non-toxic physiologically tolerable or acceptable diluents, carriers, adjuvants or vehicles that are collectively referred to herein as diluents, for parenteral injection, for oral administration in solid or liquid form, for rectal or topical administration, or the like.

The compositions can be administered to humans and animals either orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, locally, or as a buccal or nasal spray.

Compositions suitable for parenteral administration can comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into such sterile solutions or dispersions. Examples of suitable diluents include water, ethanol, polyols, suitable mixtures thereof, vegetable oils and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

Compositions can also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be insured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Besides such inert diluents, the composition can also include sweetening, flavoring and perfuming agents. Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonit, agar-agar and tragacanth, or mixtures of these substances, and the like.

The invention has been described in terms of preferred embodiments. One of ordinary skill in the art readily appreciates that

changes and modifications can be made to those embodiments without departing from the true scope of this invention.

Ins B2

INS E1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SPEAR, Patricia G.
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(ii) TITLE OF INVENTION: HERPES VIRUS ENTRY RECEPTOR PROTEIN

(iii) NUMBER OF SEQUENCES: 7

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(F) ZIP: 60601

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1719 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 293..1189

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 293..1192

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 293..406

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCTTCATACC GGCCCTTCCC CTCGGCTTTG CCTGGACAGC TCTGCCTCCC GCAGGGCCCA     60

CCTGTGTCCC CCAGCGCCGC TCCACCCAGC AGGCCTGAGC CCCTCTCTGC TGCCAGACAC    120

CCCCTGCTGC CCACTCTCCT GCTGCTCGGG TTCTGAGGCA CAGCTTGTCA CACCGAGGCG    180

GATTCTCTTT CTCTTTCTCT TCTGGCCCAC AGCCGCAGCA ATGGCGCTGA GTTCCTCTGC    240

TGGAGTTCAT CCTGCTAGCT GGGTTCCCGA GCTGCCGGTC TGAGCCTGAG GC ATG        295
                                                          Met
                                                            1

GAG CCT CCT GGA GAC TGG GGG CCT CCT CCC TGG AGA TCC ACC CCC AGA      343
Glu Pro Pro Gly Asp Trp Gly Pro Pro Pro Trp Arg Ser Thr Pro Arg
              5                  10                  15

ACC GAC GTC TTG AGG CTG GTG CTG TAT CTC ACC TTC CTG GGA GCC CCC      391
Thr Asp Val Leu Arg Leu Val Leu Tyr Leu Thr Phe Leu Gly Ala Pro
         20                  25                  30

TGC TAC GCC CCA GCT CTG CCG TCC TGC AAG GAG GAC GAG TAC CCA GTG      439
Cys Tyr Ala Pro Ala Leu Pro Ser Cys Lys Glu Asp Glu Tyr Pro Val
     35                  40                  45

GGC TCC GAG TGC TGC CCC ACG TGC AGT CCA GGT TAT CGT GTG AAG GAG      487
Gly Ser Glu Cys Cys Pro Thr Cys Ser Pro Gly Tyr Arg Val Lys Glu
 50                  55                  60                  65

GCC TGC GGG GAG CTG ACG GGC ACA GTG TGT GAA CCC TGC CCT CCA GGC      535
Ala Cys Gly Glu Leu Thr Gly Thr Val Cys Glu Pro Cys Pro Pro Gly
                 70                  75                  80

ACC TAC ATT GCC CAC CTC AAT GGC CTA AGC AAG TGT CTG CAG TGC CAA      583
Thr Tyr Ile Ala His Leu Asn Gly Leu Ser Lys Cys Leu Gln Cys Gln
             85                  90                  95

ATG TGT GAC CCA GCC ATG GGC CTG CGC GCG ACG CGG AAC TGC TCC AGG      631
Met Cys Asp Pro Ala Met Gly Leu Arg Ala Thr Arg Asn Cys Ser Arg
        100                 105                 110

ACA GAG AAC GCC GTG TGT GGC TGC AGC CCA GGC CAC TTC TGC ATC GTC      679
Thr Glu Asn Ala Val Cys Gly Cys Ser Pro Gly His Phe Cys Ile Val
    115                 120                 125

CAG GAC GGG GAC CAC TGC GCC GGT GCC GCC GTT ACG CCA CCT CCA GCC      727
Gln Asp Gly Asp His Cys Ala Gly Ala Ala Val Thr Pro Pro Pro Ala
130                 135                 140                 145

CGG GCC AGA GGG TGC AGA AGG GAG GCA CCG AGA GTC AGG ACA CCC TGT      775
Arg Ala Arg Gly Cys Arg Arg Glu Ala Pro Arg Val Arg Thr Pro Cys
                150                 155                 160

GTC AGA ACT GCC CCC GGG GAC CTT CTC TCC AAT GGG ACC CTG GAG GAA      823
Val Arg Thr Ala Pro Gly Asp Leu Leu Ser Asn Gly Thr Leu Glu Glu
            165                 170                 175

TGT CAG CAC CAG ACC AAG TGC AGC TGG CTG GTG ACG AAG GCC GGA GCT      871
Cys Gln His Gln Thr Lys Cys Ser Trp Leu Val Thr Lys Ala Gly Ala
        180                 185                 190

GGG ACC AGC AGC TCC CAC TGG GTA TGG TGG TTT CTC TCA GGG AGC CTC      919
Gly Thr Ser Ser Ser His Trp Val Trp Trp Phe Leu Ser Gly Ser Leu
    195                 200                 205

GTC ATC GTC ATT GTT TGC TCC ACA GTT GGC CTA ATC ATA TGT GTG AAA      967
Val Ile Val Ile Val Cys Ser Thr Val Gly Leu Ile Ile Cys Val Lys
210                 215                 220                 225

AGA AGA AAG CCA AGG GGT GAT GTA GTC AAG GTG ATC GTC TCC GTC CAG     1015
```

```
Arg Arg Lys Pro Arg Gly Asp Val Val Lys Val Ile Val Ser Val Gln
                230                     235                     240

CGG AAA AGA CAG GAG GCA GAA GGT GAG GCC ACA GTC ATT GAG GCC CTG      1063
Arg Lys Arg Gln Glu Ala Glu Gly Glu Ala Thr Val Ile Glu Ala Leu
            245                     250                     255

CAG GCC CCT CCG GAC GTC ACC ACG GTG GCC GTG AGG AGA CAA TAC CCT      1111
Gln Ala Pro Pro Asp Val Thr Thr Val Ala Val Arg Arg Gln Tyr Pro
        260                     265                     270

CAT TCA CGG GGA GGA GCC CAA ACC ACT GAC CCA CAG ACT CTG CAC CCC      1159
His Ser Arg Gly Gly Ala Gln Thr Thr Asp Pro Gln Thr Leu His Pro
    275                     280                     285

GAC GCC AGA GAT ACC TGG AGC GAC GGC TGC TGA AAGAGGCTGT CCACCTGGCG    1212
Asp Ala Arg Asp Thr Trp Ser Asp Gly Cys  *
290                 295                     300

AAACCACCGG AGCCCGGAGG CTTGGGGGCT CCGCCCTGGG CTGGCTTCCG TCTCCTCCAG    1272

TGGAGGGAGA GGTGGGGCCC CTGCTGGGGT AGAGCTGGGG ACGCCACGTG CCATTCCCAT    1332

GGGCCAGTGA GGGCCTGGGG CCTCTGTTCT GCTGTGGCCT GAGCTCCCCA GAGTCCTGAG    1392

GAGGAGCGCC AGTTGCCCCT CGCTCACAGA CCACACACCC AGCCCTCCTG GGCCAGCCCA    1452

GAGGGCCCTT CAGACCCCAG CTGTCTGCGC GTCTGACTCT TGTGGCCTCA GCAGGACAGG    1512

CCCCGGGCAC TGCCTCACAG CCAAGCCTGG ACTGGGTTGG CTGCAGTGTG GTGTTTAGTG    1572

GATACCACAT CGGAAGTGAT TTTCTAAATT GGATTTGAAT TCCGGTCCTG TCTTCTATTT    1632

GTCATGAAAC AGTGTATTTG GGGAGATGCT GTGGGAGGAT GTAAATATCT TGTTTCTCCT    1692

CAAAAAAAAA AAAAAAAAAA AAAAAAA                                        1719
```

Sub B2

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 299 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Pro Pro Gly Asp Trp Gly Pro Pro Pro Trp Arg Ser Thr Pro
 1               5                  10                  15

Arg Thr Asp Val Leu Arg Leu Val Leu Tyr Leu Thr Phe Leu Gly Ala
            20                  25                  30

Pro Cys Tyr Ala Pro Ala Leu Pro Ser Cys Lys Glu Asp Glu Tyr Pro
        35                  40                  45

Val Gly Ser Glu Cys Cys Pro Thr Cys Ser Pro Gly Tyr Arg Val Lys
    50                  55                  60

Glu Ala Cys Gly Glu Leu Thr Gly Thr Val Cys Glu Pro Cys Pro Pro
65                  70                  75                  80

Gly Thr Tyr Ile Ala His Leu Asn Gly Leu Ser Lys Cys Leu Gln Cys
                85                  90                  95

Gln Met Cys Asp Pro Ala Met Gly Leu Arg Ala Thr Arg Asn Cys Ser
            100                 105                 110
```

```
Arg Thr Glu Asn Ala Val Cys Gly Cys Ser Pro Gly His Phe Cys Ile
        115                 120                 125

Val Gln Asp Gly Asp His Cys Ala Gly Ala Ala Val Thr Pro Pro Pro
    130                 135                 140

Ala Arg Ala Arg Gly Cys Arg Arg Glu Ala Pro Arg Val Arg Thr Pro
145                 150                 155                 160

Cys Val Arg Thr Ala Pro Gly Asp Leu Leu Ser Asn Gly Thr Leu Glu
                165                 170                 175

Glu Cys Gln His Gln Thr Lys Cys Ser Trp Leu Val Thr Lys Ala Gly
            180                 185                 190

Ala Gly Thr Ser Ser Ser His Trp Val Trp Trp Phe Leu Ser Gly Ser
        195                 200                 205

Leu Val Ile Val Ile Val Cys Ser Thr Val Gly Leu Ile Ile Cys Val
    210                 215                 220

Lys Arg Arg Lys Pro Arg Gly Asp Val Val Lys Val Ile Val Ser Val
225                 230                 235                 240

Gln Arg Lys Arg Gln Glu Ala Glu Gly Glu Ala Thr Val Ile Glu Ala
                245                 250                 255

Leu Gln Ala Pro Pro Asp Val Thr Thr Val Ala Val Arg Arg Gln Tyr
            260                 265                 270

Pro His Ser Arg Gly Gly Ala Gln Thr Thr Asp Pro Gln Thr Leu His
        275                 280                 285

Pro Asp Ala Arg Asp Thr Trp Ser Asp Gly Cys
    290                 295
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AACCCGGCTC GAGCGGCCGC T 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAATTCCACC ACACTTAAGG TG 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACAAGACCGT TGCACCCTC

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4619 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 64..1320

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 64..1317

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
AAGCTTGCAT GCCTGCAGGT CGACTCTAGC TGGGTTCCCG AGCTGCCGGT CTGAGCCTGA        60

GGC ATG GAG CCT CCT GGA GAC TGG GGG CCT CCT CCC TGG AGA TCC ACC         108
    Met Glu Pro Pro Gly Asp Trp Gly Pro Pro Pro Trp Arg Ser Thr
      1               5                  10                  15

CCC AGA ACC GAC GTC TTG AGG CTG GTG CTG TAT CTC ACC TTC CTG GGA         156
Pro Arg Thr Asp Val Leu Arg Leu Val Leu Tyr Leu Thr Phe Leu Gly
                 20                  25                  30

GCC CCC TGC TAC GCC CCA GCT CTG CCG TCC TGC AAG GAG GAC GAG TAC         204
Ala Pro Cys Tyr Ala Pro Ala Leu Pro Ser Cys Lys Glu Asp Glu Tyr
             35                  40                  45

CCA GTG GGC TCC GAG TGC TGC CCC ACG TGC AGT CCA GGT TAT CGT GTG         252
Pro Val Gly Ser Glu Cys Cys Pro Thr Cys Ser Pro Gly Tyr Arg Val
         50                  55                  60

AAG GAG GCC TGC GGG GAG CTG ACG GGC ACA GTG TGT GAA CCC TGC CCT         300
Lys Glu Ala Cys Gly Glu Leu Thr Gly Thr Val Cys Glu Pro Cys Pro
     65                  70                  75

CCA GGC ACC TAC ATT GCC CAC CTC AAT GGC CTA AGC AAG TGT CTG CAG         348
Pro Gly Thr Tyr Ile Ala His Leu Asn Gly Leu Ser Lys Cys Leu Gln
 80                  85                  90                  95

TGC CAA ATG TGT GAC CCA GCC ATG GGC CTG CGC GCG ACG CGG AAC TGC         396
Cys Gln Met Cys Asp Pro Ala Met Gly Leu Arg Ala Thr Arg Asn Cys
                100                 105                 110

TCC AGG ACA GAG AAC GCC GTG TGT GGC TGC AGC CCA GGC CAC TTC TGC         444
Ser Arg Thr Glu Asn Ala Val Cys Gly Cys Ser Pro Gly His Phe Cys
            115                 120                 125

ATC GTC CAG GAC GGG GAC CAC TGC GCC GGT GCC GCC GTT ACG CCA CCT         492
Ile Val Gln Asp Gly Asp His Cys Ala Gly Ala Ala Val Thr Pro Pro
```

130 135 140

CCA GCC CGG GCC AGA GGG TGC AGA AGG GAG GCA CCG AGA GTC AGG ACA 540
Pro Ala Arg Ala Arg Gly Cys Arg Arg Glu Ala Pro Arg Val Arg Thr
145 150 155

CCC TGT GTC AGA ACT GCC CCC GGG GAC CTT CTC TCC AAT GGG ACC CTG 588
Pro Cys Val Arg Thr Ala Pro Gly Asp Leu Leu Ser Asn Gly Thr Leu
160 165 170 175

GAG GAA TGT CAG CAC CAG ACC AAG TGC AGA ATT CAC AAG ACC GTT GCA 636
Glu Glu Cys Gln His Gln Thr Lys Cys Arg Ile His Lys Thr Val Ala
180 185 190

CCC TCG ACA TGC AGC AAG CCC ACG TGC CCA CCC CCT GAA CTC CTG GGG 684
Pro Ser Thr Cys Ser Lys Pro Thr Cys Pro Pro Pro Glu Leu Leu Gly
195 200 205

GGA CCG TCT GTC TTC ATC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG 732
Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
210 215 220

ATC TCA CGC ACC CCC GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAG 780
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln
225 230 235

GAT GAC CCC GAG GTG CAG TTC ACA TGG TAC ATA AAC AAC GAG CAG GTG 828
Asp Asp Pro Glu Val Gln Phe Thr Trp Tyr Ile Asn Asn Glu Gln Val
240 245 250 255

CGC ACC GCC CGG CCG CCG CTA CGG GAG CAG CAG TTC AAC AGC ACG ATC 876
Arg Thr Ala Arg Pro Pro Leu Arg Glu Gln Gln Phe Asn Ser Thr Ile
260 265 270

CGC GTG GTC AGC ACC CTC CCC ATC ACG CAC CAG GAC TGG CTG AGG GGC 924
Arg Val Val Ser Thr Leu Pro Ile Thr His Gln Asp Trp Leu Arg Gly
275 280 285

Sub D2

AAG GAG TTC AAG TGC AAA GTC CAC AAC AAG GCA CTC CCG GCC CCC ATC 972
Lys Glu Phe Lys Cys Lys Val His Asn Lys Ala Leu Pro Ala Pro Ile
290 295 300

GAG AAA ACC ATC TCC AAA GCC AGA GGG CAG CCC CTG GAG CCG AAG GTC 1020
Glu Lys Thr Ile Ser Lys Ala Arg Gly Gln Pro Leu Glu Pro Lys Val
305 310 315

TAC ACC ATG GGC CCT CCC CGG GAG GAG CTG AGC AGC AGG TCG GTC AGC 1068
Tyr Thr Met Gly Pro Pro Arg Glu Glu Leu Ser Ser Arg Ser Val Ser
320 325 330 335

CTG ACC TGC ATG ATC AAC GGC TTC TAC CCT TCC GAC ATC TCG GTG GAG 1116
Leu Thr Cys Met Ile Asn Gly Phe Tyr Pro Ser Asp Ile Ser Val Glu
340 345 350

TGG GAG AAG AAC GGG AAG GCA GAG GAC AAC TAC AAG ACC ACG CCG GCC 1164
Trp Glu Lys Asn Gly Lys Ala Glu Asp Asn Tyr Lys Thr Thr Pro Ala
355 360 365

GTG CTG GAC AGC GAC GGC TCC TAC TTC CTC TAC AAC AAG CTC TCA GTG 1212
Val Leu Asp Ser Asp Gly Ser Tyr Phe Leu Tyr Asn Lys Leu Ser Val
370 375 380

CCC ACG AGT GAG TGG CAG CGG GGC GAC GTC TTC ACC TGC TCC GTG ATG 1260
Pro Thr Ser Glu Trp Gln Arg Gly Asp Val Phe Thr Cys Ser Val Met
385 390 395

CAC GAG GCC TTG CAC AAC CAC TAC ACG CAG AAG TCC ATC TCC CGC TCT 1308
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Ile Ser Arg Ser
400 405 410 415

CCG GGT AAA TGA GCGCTGTGCC GGCGAGCTGC CCTCTCCCT CCCCCCCACG 1360
Pro Gly Lys *

CCGCAGCTGT GCACCCCGCA CACAAATAAA GCACCCAGCT CTGCCCTGAA CAGCTTCCGG 1420

TCTCCCTATA GTGAGTCGTA TTAATTTCGA TAAGCCAGCT GCATTAATGA ATCGGCCAAC 1480

GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC 1540

TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT 1600

TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG 1660

CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG 1720

AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT 1780

ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA 1840

CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT 1900

GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC 1960

CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA 2020

GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG 2080

TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG 2140

TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT 2200

GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA 2260

CGCGCAGAAA AAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC 2320

AGTGGAACGA AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA 2380

CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA 2440

CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT 2500

Sub D

TTCGTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT 2560

TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT 2620

TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT 2680

CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA 2740

ATAGTTTGCG CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG 2800

GTATGGCTTC ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT 2860

TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG 2920

CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG 2980

TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC 3040

GGCGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA 3100

CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC 3160

CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT 3220

TTACTTTCAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG 3280

GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA 3340

GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA 3400
AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA 3460
TTATTATCAT GACATTAACC TATAAAAATA GGCGTATCAC GAGGCCCTTT CGTCTCGCGC 3520
GTTTCGGTGA TGACGGTGAA AACCTCTGAC ACATGCAGCT CCCGGAGACG GTCACAGCTT 3580
GTCTGTAAGC GGATGCCGGG AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG 3640
GGTGTCGGGG CTGGCTTAAC TATGCGGCAT CAGAGCAGAT TGTACTGAGA GTGCACCATA 3700
TCGACGCTCT CCCTTATGCG ACTCCTGCAT TAGGAAGCAG CCCAGTAGTA GGTTGAGGCC 3760
GTTGAGCACC GCCGCCGCAA GGAATGGTGC AAGGAGATGG CGCCCAACAG TCCCCCGGCC 3820
ACGGGGCCTG CCACCATACC CACGCCGAAA CAAGCGCTCA TGAGCCCGAA GTGGCGAGCC 3880
CGATCTTCCC CATCGGTGAT GTCGGCGATA TAGGCGCCAG CAACCGCACC TGTGGCGCCG 3940
GTGATGCCGG CCACGATGCG TCCGGCGTAG AGGATCTGGC TAGTTATTAA TAGTAATCAA 4000
TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG CGTTACATAA CTTACGGTAA 4060
ATGGCCCGCC TGGCTGACCG CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG 4120
TTCCCATAGT AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAC TATTTACGGT 4180
AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG 4240
TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA TGGGACTTTC 4300
CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC CATGGTGATG CGGTTTTGGC 4360
AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA 4420
TTGACGTCAA TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA 4480
ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA 4540
GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTAACT GGCTTATCGA AATTAATACG 4600
ACTCACTATA GGGAGACCC 4619

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 418 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Glu Pro Pro Gly Asp Trp Gly Pro Pro Pro Trp Arg Ser Thr Pro
 1               5                  10                  15

Arg Thr Asp Val Leu Arg Leu Val Leu Tyr Leu Thr Phe Leu Gly Ala
            20                  25                  30

Pro Cys Tyr Ala Pro Ala Leu Pro Ser Cys Lys Glu Asp Glu Tyr Pro
        35                  40                  45

Val Gly Ser Glu Cys Cys Pro Thr Cys Ser Pro Gly Tyr Arg Val Lys
    50                  55                  60

Glu Ala Cys Gly Glu Leu Thr Gly Thr Val Cys Glu Pro Cys Pro Pro
```

65 70 75 80

Gly Thr Tyr Ile Ala His Leu Asn Gly Leu Ser Lys Cys Leu Gln Cys
85 90 95

Gln Met Cys Asp Pro Ala Met Gly Leu Arg Ala Thr Arg Asn Cys Ser
100 105 110

Arg Thr Glu Asn Ala Val Cys Gly Cys Ser Pro Gly His Phe Cys Ile
115 120 125

Val Gln Asp Gly Asp His Cys Ala Gly Ala Ala Val Thr Pro Pro Pro
130 135 140

Ala Arg Ala Arg Gly Cys Arg Arg Glu Ala Pro Arg Val Arg Thr Pro
145 150 155 160

Cys Val Arg Thr Ala Pro Gly Asp Leu Leu Ser Asn Gly Thr Leu Glu
165 170 175

Glu Cys Gln His Gln Thr Lys Cys Arg Ile His Lys Thr Val Ala Pro
180 185 190

Ser Thr Cys Ser Lys Pro Thr Cys Pro Pro Pro Glu Leu Leu Gly Gly
195 200 205

Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
210 215 220

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Asp
225 230 235 240

Asp Pro Glu Val Gln Phe Thr Trp Tyr Ile Asn Asn Glu Gln Val Arg
245 250 255

Thr Ala Arg Pro Pro Leu Arg Glu Gln Gln Phe Asn Ser Thr Ile Arg
260 265 270

Val Val Ser Thr Leu Pro Ile Thr His Gln Asp Trp Leu Arg Gly Lys
275 280 285

Glu Phe Lys Cys Lys Val His Asn Lys Ala Leu Pro Ala Pro Ile Glu
290 295 300

S4 D2

Lys Thr Ile Ser Lys Ala Arg Gly Gln Pro Leu Glu Pro Lys Val Tyr
305 310 315 320

Thr Met Gly Pro Pro Arg Glu Glu Leu Ser Ser Arg Ser Val Ser Leu
325 330 335

Thr Cys Met Ile Asn Gly Phe Tyr Pro Ser Asp Ile Ser Val Glu Trp
340 345 350

Glu Lys Asn Gly Lys Ala Glu Asp Asn Tyr Lys Thr Thr Pro Ala Val
355 360 365

Leu Asp Ser Asp Gly Ser Tyr Phe Leu Tyr Asn Lys Leu Ser Val Pro
370 375 380

Thr Ser Glu Trp Gln Arg Gly Asp Val Phe Thr Cys Ser Val Met His
385 390 395 400

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Ile Ser Arg Ser Pro
405 410 415

Gly Lys

WHAT IS CLAIMED IS:

1. An isolated and purified polypeptide of about 300 amino acid residues comprising the amino acid residue sequence ~~SEQ ID~~ NO:2.

2. A recombinant human HVER.

3. A process of detecting an antibody against HVER in a biological sample comprising adding the polypeptide of claim 1 to the sample, maintaining the sample for a period of time sufficient for formation of a conjugate between the antibody and the polypeptide and detecting the presence of the conjugate and thereby the antibody.

Amb A1

4. An isolated and purified polynucleotide comprising a nucleotide sequence consisting essentially of a nucleotide sequence selected from the group consisting of: a) the sequence of SEQ ID NO:1 from nucleotide position 293 to about nucleotide position 1189; b) sequences that are complementary to the sequence of (a); c) sequences that, on expression, encode a polypeptide encoded by the sequence of (a).

C1

5. The polynucleotide of claim 4 that is a DNA molecule.

Sub B4

6. The polynucleotide of claim 5 wherein ~~the nucleotide~~ sequence ~~is SEQ~~ ID NO:1.

7. The polynucleotide of claim 4 that is an RNA molecule.

8. An expression vector comprising the DNA molecule of claim 5.

9. The expression vector of claim 8 further comprising an enhancer-promoter operatively linked to the polynucleotide.

Sub B5

10. The expression vector of claim 8 wherein the polynucleotide has the nucleotide sequence of SEQ ID NO:1 from nucleotide position 293 to about nucleotide position 1189.

11. An oligonucleotide of from about 15 to about 50 nucleotides containing a nucleotide sequence of at least 15 nucleotides that is identical or complementary to a contiguous sequence of the polynucleotide of claim 4.

12. The oligonucleotide of claim 11 that is an antisense oligonucleotide.

13. A host cell transformed with the expression vector of claim 8.

14. The transformed host cell of claim 13 that is a mammalian cell.

15. The transformed host cell of claim 13 that is a bacterial cell.

16. The transformed host cell of claim 14 wherein the mammalian cell is an ovarian cell.

17. The transformed host cell of claim 16 wherein the ovarian cell is designated CHO-A3, CHO-A12, CHO-B3, CHO-B9, or CHO-B11.

18. A process of making HVER comprising transforming a host cell with the expression vector of claim 8, maintaining the transformed cell for a period of time sufficient for expression of the HVER and recovering the HVER.

19. The process of claim 18 wherein the host cell is an eukaryotic host cell.

15 ~~20.~~ The process of claim ~~19~~ 14 wherein the host cell is a mammalian cell.

21. The process of claim 20 wherein the mammalian cell is an ovarian cell.

22. The process of claim 18 wherein the HVER is human HVER.

23. The process of claim 18 wherein ~~the polynucleotide~~ has the nucleotide sequence of SEQ ~~ID~~ NO:1 from nucleotide position 293 to about ~~nucleotide~~ position 1189.

24. HVER made by the process of ~~claim 18.~~

25. A pharmaceutical composition comprising the oligonucleotide of claim 12 and a physiological acceptable diluent.

26. A pharmaceutical composition comprising the polypeptide of claim ~~1 and a physiologically~~ acceptable diluent.

27. A plasmid selected from the group consisting of pBEC10, pBEC580, and pBL58.

Add C1

Add E6

Abstract of the Disclosure

08/509024

The present invention provides isolated and purified polynucleotides that encode HVEM ~~HVER~~ of mammalian origin, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process of making HVEM ~~HVER~~ using those polynucleotides and vectors, and isolated and purified HVEM ~~HVER~~.

7/27/98 .XESS MAILING LABEL # EF420572989US

Applicant or Patentee: Spear~et
Serial or Patent No.: Not yet assigned
Filed or Issued: herewith
For: HERPES VIRUS ENTRY RECEPTOR PROTEIN

PATENT

Atty Docket No. NOR3446P001C

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS

(37 C.F.R. 1.9(f) AND 1.27(d) - NONPROFIT ORGANIZATION)

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: NORTHWESTERN UNIVERSITY
ADDRESS OF ORGANIZATION: 1801 Maple, Evanston, IL 60201

TYPE OF ORGANIZATION:

[x] UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
[ ] TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3)
[ ] NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE ______)
(CITATION OF STATUTE ______)
[ ] WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3)) IF LOCATED IN UNITED STATES OF AMERICA
[ ] WOULD QUALIFY AS NON PROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE ______)
(CITATION OF STATUTE ______)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees under Section 41(z) and (b) of Title 35, United States Code with regard to the invention entitled ______ by inventor(s) ______ described in:

[x] the specification filed herewith.
[ ] Application Serial No. ______, filed ______.
[ ] Patent No. ______, issued ______.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the above-identified nonprofit organization are not exclusive, each individual, concern, or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. 1.9(d), or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. 1.27)

NAME ______
ADDRESS ______
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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

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PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY

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My residence, post office address, and citizenship are as stated below.

I believe I am the original, first, and sole inventor (if only one name is listed) or an original, first, and joint inventor (if plural names are listed) of the subject matter which is claimed and for which a patent is sought on the invention entitled: HERPES VIRUS ENTRY RECEPTOR PROTEIN the specification of which:

x is attached hereto;

____ was filed on ________ as Application Serial No. ________ and was amended on ________ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to herein.

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I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Todd M. Crissey	Reg. No. 37,807	Gerson E. Meyers	Reg. No. 21,160	Joel E. Siegel	Reg. No. 25,440
Karl R. Fink	Reg. No. 34,161	John P. Milnamow	Reg. No. 20,635	Paul M. Vargo	Reg. No. 29,116
Stephen D. Geimer	Reg. No. 28,846	Thomas E. Northrup	Reg. No. 33,268	Mitchell J. Weinstein	Reg. No. 37,963
Allen J. Hoover	Reg. No. 24,103				

whose mailing address for this application is:

DRESSLER, GOLDSMITH, SHORE & MILNAMOW, LTD.
Two Prudential Plaza - Suite 4700
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Chicago, Illinois 60601
Telephone: (312) 616-5400

1-00

Full name of SOLE or FIRST inventor Patricia G. Spear
Citizenship U.S.A. Residence 5719 S. Kenwood Avenue
Chicago, IL 60637
Post Office Address (If different) ________

Inventor's signature: ________ Date: ________

Full name of SECOND joint inventor, if any Rebecca I. Montgomery
Citizenship U.S.A. Residence 603 The Lane
Hinsdale, IL 60521
Post Office Address (If different) ________

Second Inventor's signature: ________ Date: ________

Full name of THIRD joint inventor, if any ________
Citizenship ________ Residence ________
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(Signature of person mailing paper or fee)

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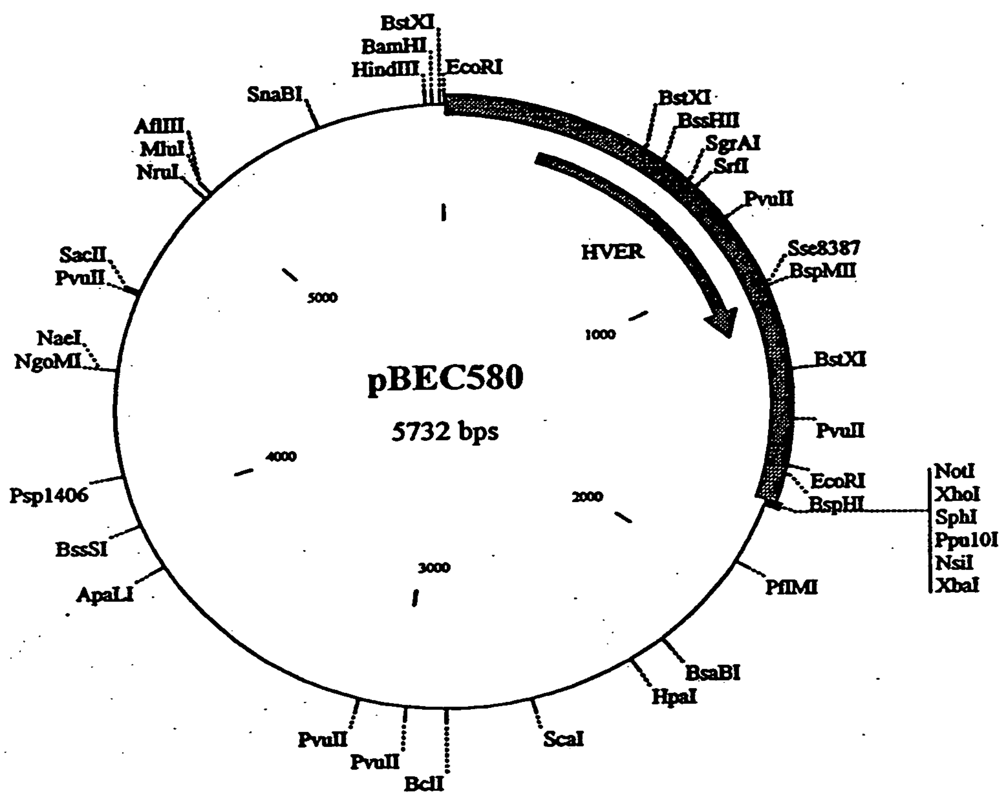

FIG. 1

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  61  CCTGTGTCCC C[illegible]GCCGC TCCACCCAGC AGGCCTGAGC CCC[illegible]TCTGC TGCCAGACAC
 121  CCCCTGCTGC CCACTCTCCT GCTGCTCGGG TTCTGAGGCA CAGCTTGTCA CACCGAGGCG
 181  GATTCTCTTT CTCTTTCTCT TCTGGCCCAC AGCCGCAGCA ATGGCGCTGA GTTCCTCTGC
 241  TGGAGTTCAT CCTGCTAGCT GGGTTCCCGA GCTGCCGGTC TGAGCCTGAG GCATGGAGCC
   1                                                          M  E  P

 301  TCCTGGAGAC TGGGGGCCTC CTCCCTGGAG ATCCACCCCC AGAACCGACG TCTTGAGGCT
   4   P  G  D   W  G  P   P  P  W  R   S  T  P   R  T  D   V  L  R  L

 361  GGTGCTGTAT CTCACCTTCC TGGGAGCCCC CTGCTACGCC CCAGCTCTGC CGTCCTGCAA
  24   V  L  Y   L  T  F   L  G  A  P   C  Y  A   P  A  L   P  S  C  K

 421  GGAGGACGAG TACCCAGTGG GCTCCGAGTG CTGCCCCACG TGCAGTCCAG GTTATCGTGT
  44   E  D  E   Y  P  V   G  S  E  C   C  P  T   C  S  P   G  Y  R  V

 481  GAAGGAGGCC TGCGGGGAGC TGACGGGCAC AGTGTGTGAA CCCTGCCCTC CAGGCACCTA
  64   K  E  A   C  G  E   L  T  G  T   V  C  E   P  C  P   P  G  T  Y

 541  CATTGCCCAC CTCAATGGCC TAAGCAAGTG TCTGCAGTGC CAAATGTGTG ACCCAGCCAT
  84   I  A  H   L  N  G   L  S  K  C   L  Q  C   Q  M  C   D  P  A  M

 601  GGGCCTGCGC GCGACGCGGA ACTGCTCCAG GACAGAGAAC GCCGTGTGTG GCTGCAGCCC
 104   G  L  R   A  T  R   N  C  S  R   T  E  N   A  V  C   G  C  S  P

 661  AGGCCACTTC TGCATCGTCC AGGACGGGGA CCACTGCGCC GGTGCCGCCG TTACGCCACC
 124   G  H  F   C  I  V   Q  D  G  D   H  C  A   G  A  A   V  T  P  P

 721  TCCAGCCCGG GCCAGAGGGT GCAGAAGGGA GGCACCGAGA GTCAGGACAC CCTGTGTCAG
 144   P  A  R   A  R  G   C  R  R  E   A  P  R   V  R  T   P  C  V  R

 781  AACTGCCCCC GGGGACCTTC TCTCCAATGG GACCCTGGAG GAATGTCAGC ACCAGACCAA
 164   T  A  P   G  D  L   L  S  N  G   T  L  E   E  C  Q   H  Q  T  K

 841  GTGCAGCTGG CTGGTGACGA AGGCCGGAGC TGGGACCAGC AGCTCCCACT GGGTATGGTG
 184   C  S  W   L  V  T   K  A  G  A   G  T  S   S  S  H   W  V  W  W

 901  GTTTCTCTCA GGGAGCCTCG TCATCGTCAT TGTTTGCTCC ACAGTTGGCC TAATCATATG
 204   F  L  S   G  S  L   V  I  V  I   V  C  S   T  V  G   L  I  I  C

 961  TGTGAAAAGA AGAAAGCCAA GGGGTGATGT AGTCAAGGTG ATCGTCTCCG TCCAGCGGAA
 224   V  K  R   R  K  P   R  G  D  V   V  K  V   I  V  S   V  Q  R  K

1021  AAGACAGGAG GCAGAAGGTG AGGCCACAGT CATTGAGGCC CTGCAGGCCC CTCCGGACGT
 244   R  Q  E   A  E  G   E  A  T  V   I  E  A   L  Q  A   P  P  D  V

1081  CACCACGGTG GCCGTGAGGA GACAATACCC TCATTCACGG GGAGGAGCCC AAACCACTGA
 264   T  T  V   A  V  R   R  Q  Y  P   H  S  R   G  G  A   Q  T  T  D

1141  CCCACAGACT CTGCACCCCG ACGCCAGAGA TACCTGGAGC GACGGCTGCT GAAAGAGGCT
 284   P  Q  T   L  H  P   D  A  R  D   T  W  S   D  G  C

1201  GTCCACCTGG CGAAACCACC GGAGCCCGGA GGCTTGGGGG CTCCGCCCTG GGCTGGCTTC
1261  CGTCTCCTCC AGTGGAGGGA GAGGTGGGGC CCCTGCTGGG GTAGAGCTGG GGACGCCACG
1321  TGCCATTCCC ATGGGCCAGT GAGGGCCTGG GGCCTCTGTT CTGCTGTGGC CTGAGCTCCC
1381  CAGAGTCCTG AGGAGGAGCG CCAGTTGCCC CTCGCTCACA GACCACACAC CCAGCCCTCC
1441  TGGGCCAGCC CAGAGGGCCC TTCAGACCCC AGCTGTCTGC GCGTCTGACT CTTGTGGCCT
1501  CAGCAGGACA GGCCCCGGGC ACTGCCTCAC AGCCAAGGCT GGACTGGGTT GGCTGCAGTG
1561  TGGTGTTTAG TGGATACCAC ATCGGAAGTG ATTTTCTAAA TTGGATTTGA ATTCCGGTCC
1621  TGTCTTCTAT TTGTCATGAA ACAGTGTATT TGGGGAGATG CTGTGGGAGG ATGTAAATAT
1681  CTTGTTTCTC CTCAAAAAAA AAAAAAAAAA AAAAAAAAA
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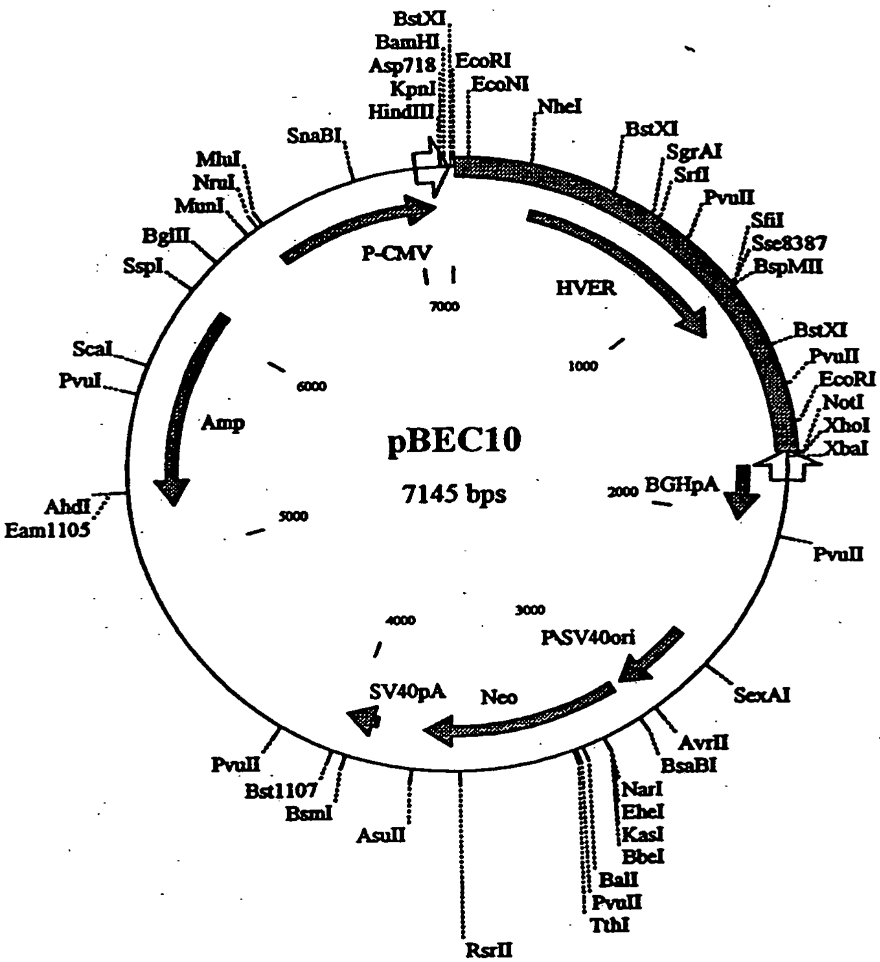
FIG. 2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EcoRI
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SrfI
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SfiI
Sse8387
BspMII
BstXI
PvuII
EcoRI
NotI
XhoI
XbaI
PvuII
SexAI
AvrII
BsaBI
NarI
EheI
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NruI
MluI
SnaBI
P-CMV
HVER
BGHpA
PSV40ori
Neo
SV40pA
Amp
pBEC10
7145 bps
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1000
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3000
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5000
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FIG. 3

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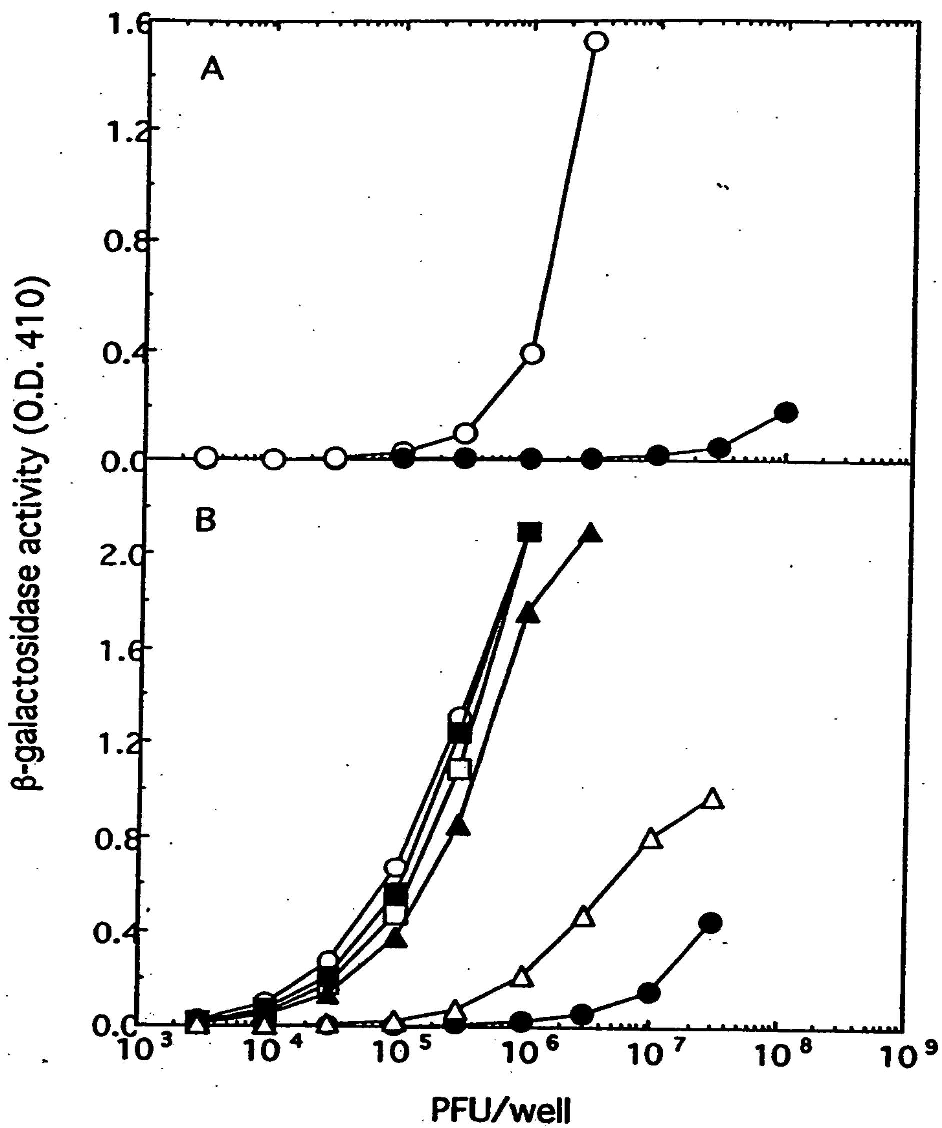

FIG. 4

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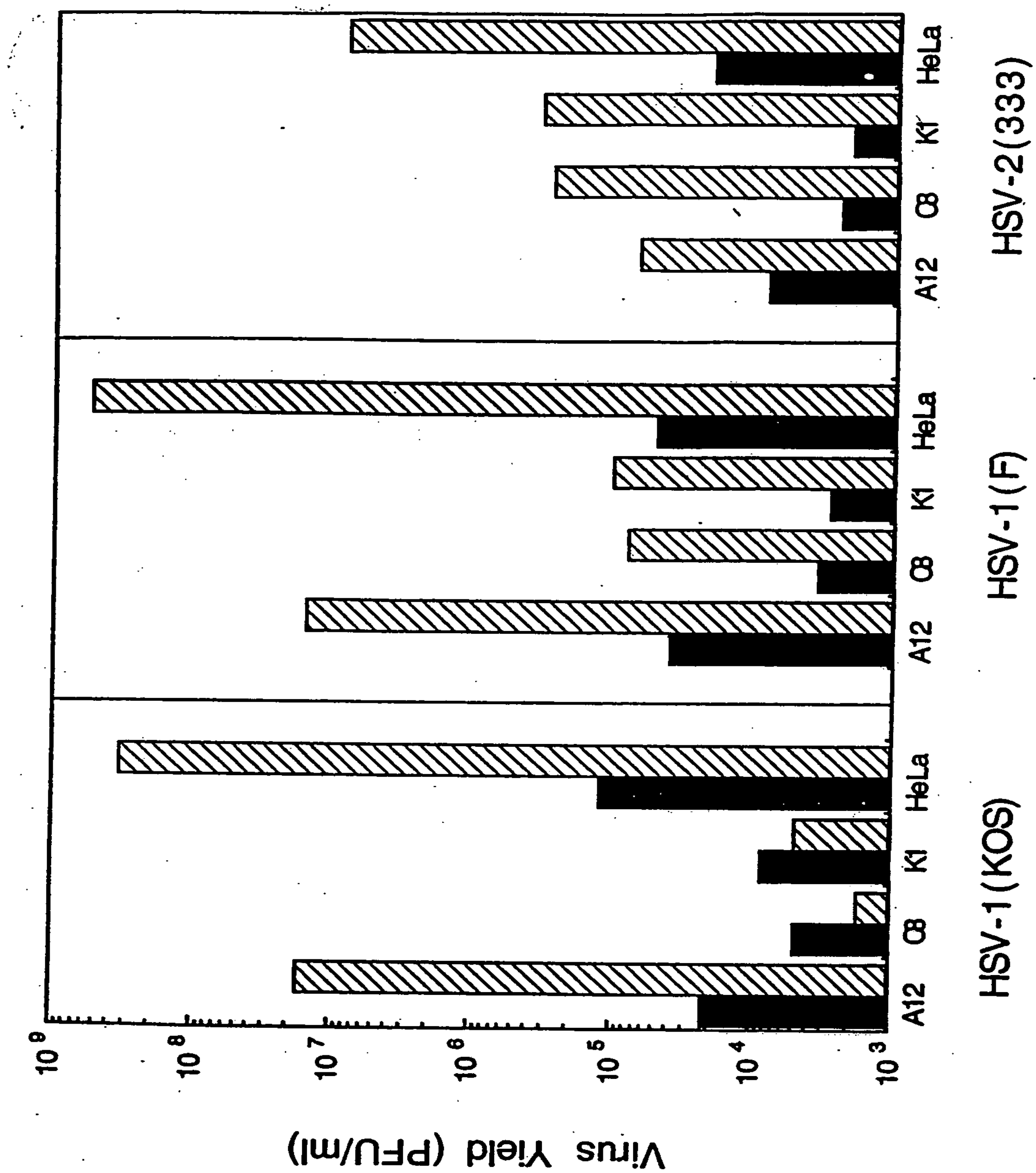

FIG. 5

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gD-1 and gD-2 interfere with KOS(gL86) infection of A12 cells

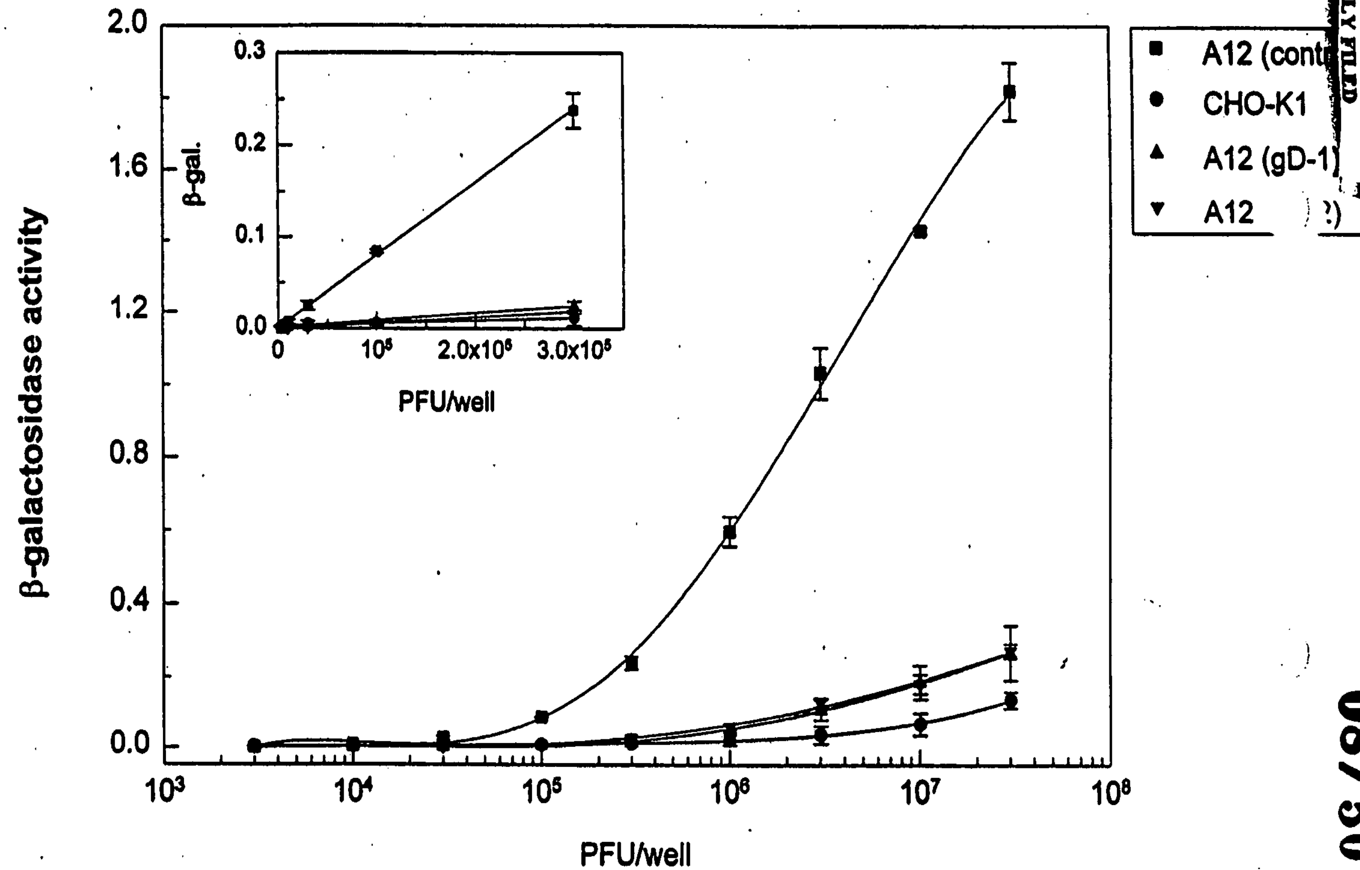

FIG. 6

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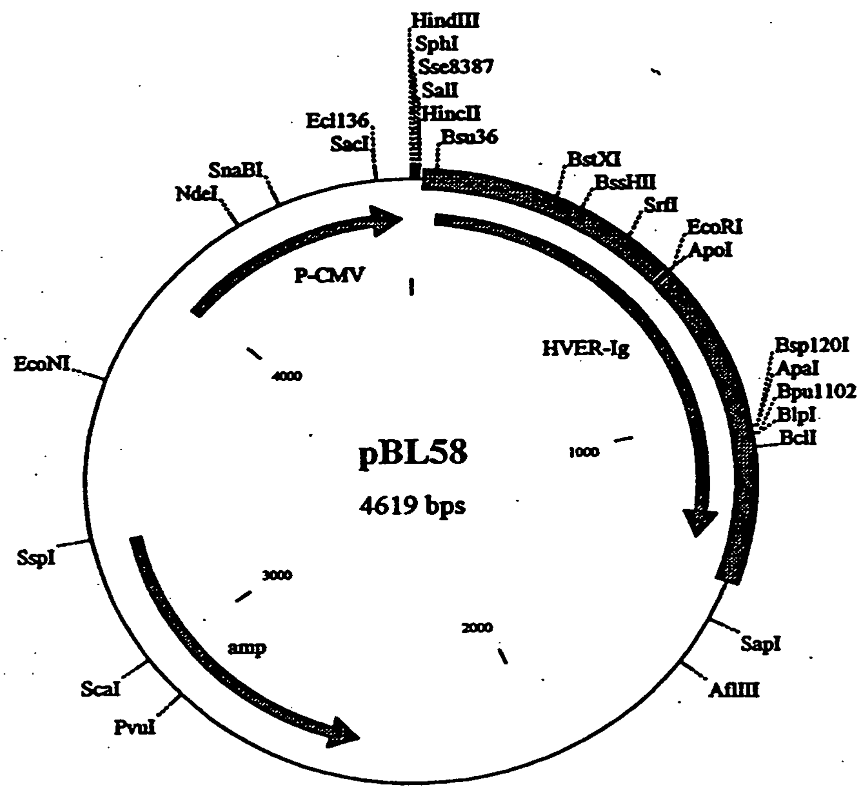

FIG. 7

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   1  AAGCTTGCAT GC    AGGT CGACTCTAGC TGGGTTCCCG AG     CGGT CTGAGCCTGA
  61  GGCATGGAGC C     GGAGA CTGGGGGCCT CCTCCCTGGA G      CCCC CAGAACCGAC
   1    M  E  I     G  D   W  G  P   P  P  W   R    T  P   R  T  D

 121  GTCTTGAGGC TGGTGCTGTA TCTCACCTTC CTGGGAGCCC CCTGCTACGC CCCAGCTCTG
  20   V  L  R   L  V  L  Y   L  T  F   L  G  A   P  C  Y  A  P  A  L

 181  CCGTCCTGCA AGGAGGACGA GTACCCAGTG GGCTCCGAGT GCTGCCCCAC GTGCAGTCCA
  40   P  S  C   K  E  D  E   Y  P  V   G  S  E   C  C  P  T   C  S  P

 241  GGTTATCGTG TGAAGGAGGC CTGCGGGGAG CTGACGGGCA CAGTGTGTGA ACCCTGCCCT
  60   G  Y  R   V  K  E  A   C  G  E   L  T  G   T  V  C  E   P  C  P

 301  CCAGGCACCT ACATTGCCCA CCTCAATGGC CTAAGCAAGT GTCTGCAGTG CCAAATGTGT
  80   P  G  T   Y  I  A  H   L  N  G   L  S  K   C  L  Q  C   Q  M  C

 361  GACCCAGCCA TGGGCCTGCG CGCGACGCGG AACTGCTCCA GGACAGAGAA CGCCGTGTGT
 100   D  P  A   M  G  L  R   A  T  R   N  C  S   R  T  E  N   A  V  C

 421  GGCTGCAGCC CAGGCCACTT CTGCATCGTC CAGGACGGGG ACCACTGCGC CGGTGCCGCC   136 -
 120   G  C  S   P  G  H  F   C  I  V   Q  D  G   D  H  C  A   G  A  A   172

 481  GTTACGCCAC CTCCAGCCCG GGCCAGAGGG TGCAGAAGGG AGGCACCGAG AGTCAGGACA
 140   V  T  P   P  P  A  R   A  R  G   C  R  R   E  A  P  R   V  R  T

 541  CCCTGTGTCA GAACTGCCCC CGGGGACCTT CTCTCCAATG GGACCCTGGA GGAATGTCAG
 160   P  C  V   R  T  A  P   G  D  L   L  S  N   G  T  L  E   E  C  Q

 601  CACCAGACCA AGTGCAGAAT TCACAAGACC GTTGCACCCT CGACATGCAG CAAGCCCACG
 180   H  Q  T   K  C  R  I   H  K  T   V  A  P   S  T  C  S   K  P  T

 661  TGCCCACCCC CTGAACTCCT GGGGGGACCG TCTGTCTTCA TCTTCCCCCC AAAACCCAAG
 200   C  P  P   P  E  L  L   G  G  P   S  V  F   I  F  P  P   K  P  K

 721  GACACCCTCA TGATCTCACG CACCCCCGAG GTCACATGCG TGGTGGTGGA CGTGAGCCAG
 220   D  T  L   M  I  S  R   T  P  E   V  T  C   V  V  V  D   V  S  Q

 781  GATGACCCCG AGGTGCAGTT CACATGGTAC ATAAACAACG AGCAGGTGCG CACCGCCCGG
 240   D  D  P   E  V  Q  F   T  W  Y   I  N  N   E  Q  V  R   T  A  R

 841  CCGCCGCTAC GGGAGCAGCA GTTCAACAGC ACGATCCGCG TGGTCAGCAC CCTCCCCATC
 260   P  P  L   R  E  Q  Q   F  N  S   T  I  R   V  V  S  T   L  P  I

 901  ACGCACCAGG ACTGGCTGAG GGGCAAGGAG TTCAAGTGCA AAGTCCACAA CAAGGCACTC
 280   T  H  Q   D  W  L  R   G  K  E   F  K  C   K  V  H  N   K  A  L

 961  CCGGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAGAGGGC AGCCCCTGGA GCCGAAGGTC
 300   P  A  P   I  E  K  T   I  S  K   A  R  G   Q  P  L  E   P  K  V

1021  TACACCATGG GCCCTCCCCG GGAGGAGCTG AGCAGCAGGT CGGTCAGCCT GACCTGCATG
 320   Y  T  M   G  P  P  R   E  E  L   S  S  R   S  V  S  L   T  C  M

1081  ATCAACGGCT TCTACCCTTC CGACATCTCG GTGGAGTGGG AGAAGAACGG GAAGGCAGAG
 340   I  N  G   F  Y  P  S   D  I  S   V  E  W   E  K  N  G   K  A  E

1141  GACAACTACA AGACCACGCC GGCCGTGCTG GACAGCGACG GCTCCTACTT CCTCTACAAC
 360   D  N  Y   K  T  T  P   A  V  L   D  S  D   G  S  Y  F   L  Y  N

1201  AAGCTCTCAG TGCCCACGAG TGAGTGGCAG CGGGGCGACG TCTTCACCTG CTCCGTGATG
 380   K  L  S   V  P  T  S   E  W  Q   R  G  D   V  F  T  C   S  V  M

1261  CACGAGGCCT TGCACAACCA CTACACGCAG AAGTCCATCT CCCGCTCTCC GGGTAAATGA
 400   H  E  A   L  H  N  H   Y  T  Q   K  S  I   S  R  S  P   G  K  -
```

FIG 8A

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1321 GCGCTGTGCC GG[illegible]TGC CCCTCTCCCT CCCCCCCACG CCG[illegible]TGT GCACCCCGCA
1381 CACAAATAAA GCA[illegible]AGCT CTGCCCTGAA CAGCTTCCGG TCT[illegible]ATA GTGAGTCGTA
1441 TTAATTTCGA TAAGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG
1501 CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG
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1621 AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC
1681 GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC
1741 TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA
1801 AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT
1861 CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG
1921 TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC
1981 GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG
2041 GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC
2101 TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG
2161 CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC
2221 GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT
2281 CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT
2341 TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA
2401 AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA
2461 TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC
2521 TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT
2581 GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA
2641 GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT
2701 AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG CAACGTTGTT
2761 GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC
2821 GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC
2881 TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT
2941 ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT
3001 GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC
3061 CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT
3121 GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCG
3181 ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC CAGCGTTTCT
3241 GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA
3301 TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT
3361 CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC
3421 ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA TTATTATCAT GACATTAACC
3481 TATAAAAATA GGCGTATCAC GAGGCCCTTT CGTCTCGCGC GTTTCGGTGA TGACGGTGAA
3541 AACCTCTGAC ACATGCAGCT CCCGGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG
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3721 ACTCCTGCAT TAGGAAGCAG CCCAGTAGTA GGTTGAGGCC GTTGAGCACC GCCGCCGCAA
3781 GGAATGGTGC AAGGAGATGG CGCCCAACAG TCCCCCGGCC ACGGGGCCTG CCACCATACC
3841 CACGCCGAAA CAAGCGCTCA TGAGCCCGAA GTGGCGAGCC CGATCTTCCC CATCGGTGAT
3901 GTCGGCGATA TAGGCGCCAG CAACCGCACC TGTGGCGCCG GTGATGCCGG CCACGATGCG
3961 TCCGGCGTAG AGGATCTGGC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT
4021 AGCCCATATA TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
4081 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA
4141 GGGACTTTCC ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA
4201 CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC
4261 GCCTGGCATT ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC
4321 GTATTAGTCA TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
4381 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG
4441 TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG
4501 CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT
4561 AGAGAACCCA CTGCTTAACT GGCTTATCGA AATTAATACG ACTCACTATA GGGAGACCC

FIG. 8B

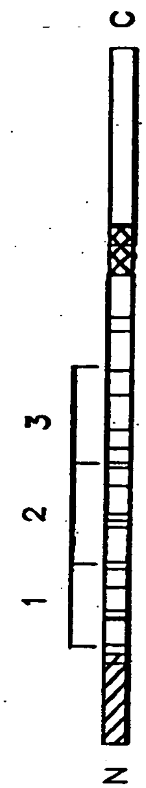

FIG. 9

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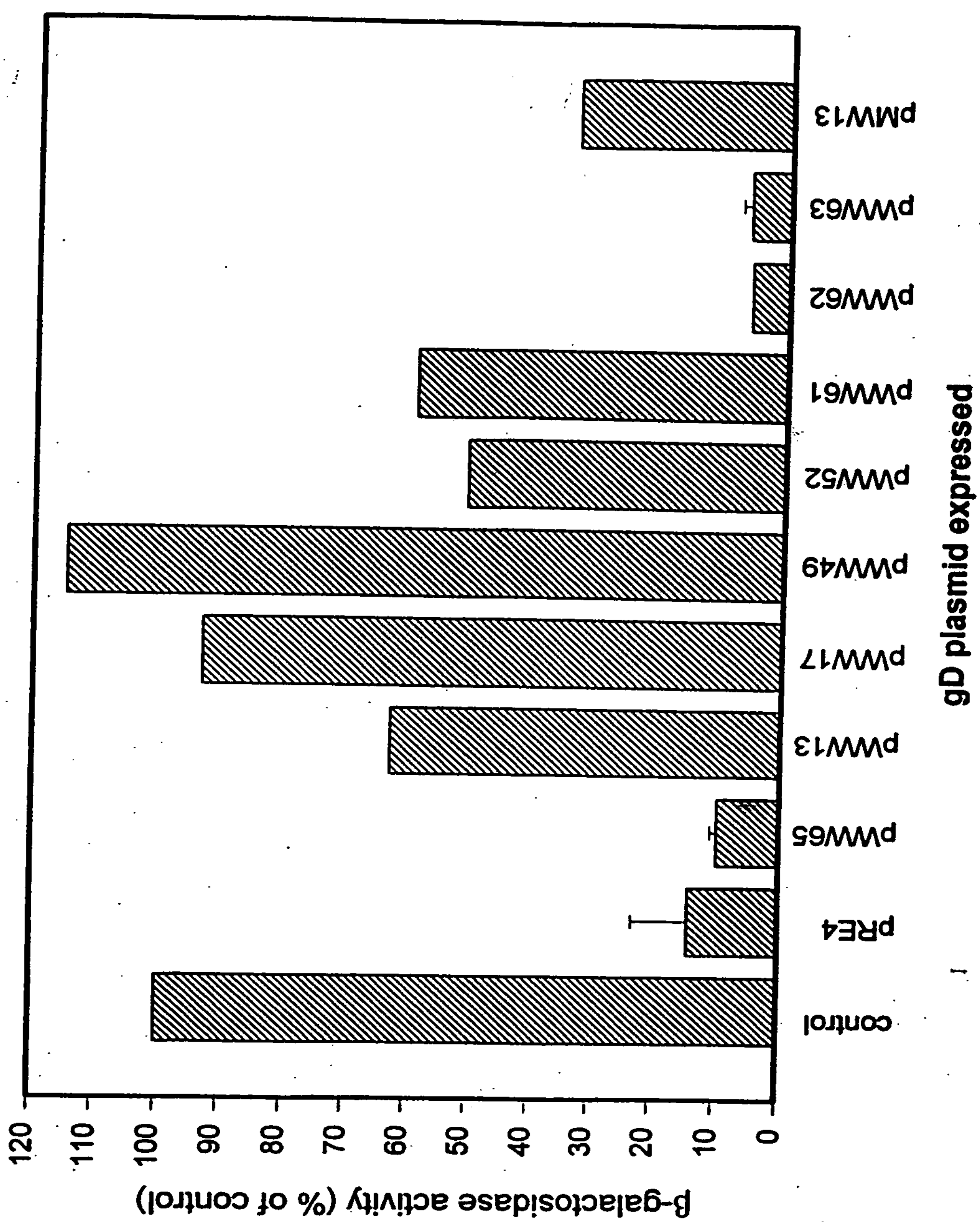

FIG. 10